CANADIAN JOURNAL OF RESEARCH

VOLUME 21 JANUARY, 1943 NUMBER 1

CONTENTS

NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

Publications and Subscriptions

The Canadian Journal of Research is issued monthly in four sections, as follows:

A. Physical Sciences
B. Chemical Sciences
C. Botanical Sciences
D. Zoological Sciences

For the present, Sections A and B are issued under a single cover, as also are Sections C and D, with separate pagination of the four sections, to permit separate binding, if desired.

Subscription rates, postage paid to any part of the world are as follows:

	Annual	*Single Copy*
A and B	$ 2.50	$ 0.50
C and D	2.50	0.50
Four sections, complete	4.00	—

The Canadian Journal of Research is published by the National Research Council of Canada under authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. All correspondence should be addressed:

National Research Council, Ottawa, Canada.

Notice to Contributors

Fifty reprints of each paper are supplied free. Additional reprints, if required, will be supplied according to a prescribed schedule of charges.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 21, SEC. C. JANUARY, 1943 NUMBER 1

A RING SPOT DISEASE OF GLADIOLUS CORMS[1]

BY COLIN D. MCKEEN[2]

Abstract

A ring spot disease of gladiolus, confined to the corm and manifested only in storage, has been observed at Vineland, Ont. It is characterized by rather superficial, reddish-brown lesions walled off from healthy tissue by a periderm layer and bearing somewhat conspicuous concentric rings originating at the nodes and around the root initials.

The progeny of diseased corms are almost invariably diseased, ring spots developing about two and one-half months after harvesting. The disease development or the nature of the symptoms was not modified to any appreciable extent by various storage temperatures and relative humidities.

Infrequently from small lesions and more often from large ones *Penicillium* and *Fusarium* isolates were obtained. Inoculations with these organisms yielded negative results. The cause of the disease has therefore not been established.

No control for the disease has been effected by treating the corms with various disinfectants.

A previously undescribed disease of gladiolus has been noticed recently in Ontario. In certain varieties this disease has rendered an appreciable number of corms unfit for sale as propagative lines and thus it seems likely to become important economically.

Symptoms of the Disease

The symptoms are confined to the corm and are manifested during the storage period. The most characteristic symptom is the presence of lesions on the corm bearing somewhat conspicuous, irregularly concentric rings or markings. The centre of the system of rings, which constitute a lesion, is located on a node (Fig. 1) or a root initial. The successive rings may be very close together or somewhat separated, the condition varying from variety to variety. If close together, the concentric rings appear as narrow, dark reddish-brown zones over the lighter brown background of the affected region. If the rings are more remote, they are wider and occasionally there is a narrow band of healthy tissue between them (Fig. 2). These ring spots occur more frequently on the lower half of the corm, but are by no means restricted to that area. Occasionally in badly affected specimens an equal number of ring

[1] *Manuscript received November 9, 1942.*

Contribution from the Department of Botany, University of Toronto, Toronto, Ont. Based on a thesis presented in May, 1942, to the University of Toronto in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[2] *Class Assistant, Department of Botany, University of Toronto.*

spots is present on the upper half of the corm. To determine assuredly the presence of the disease it is necessary to remove the leaf scales from the corm. In many instances, however, when ring spots are present there is also a discoloration of the husks accompanied by a loss of lustre (Fig. 3).

There is no evidence of the disease at the time of harvesting in late autumn, and only after the corms have been in storage some time do the symptoms appear. The first detectable symptom is a change in colour of the corm in small localized areas approximately 1 to 2 mm. in diameter at the nodes and root initials. This may constitute the innermost ring of the lesion and after some time another concentric ring may appear on a greater radius leaving, frequently, a band of healthy tissue between the two. Occasionally, no such tissue is contained within the lesion and the rings appear as darker reddish-brown zones in the affected areas. The ring spots may continue to advance until lesions from contiguous nodes fuse in the internodal regions (Fig. 4). In severely infected corms, where the centres of infection are close together, there is frequently a fusion of these along the node to form a continuous band around the corm before the ring spots from adjacent nodes coalesce in the internodal area.

At an early period the infected areas are slightly elevated on the surface of the corm. Quite often, when the lesions are deep, these discoloured elevated areas may become slightly depressed after diseased corms have been in storage five or more months. Most of the lesions are essentially superficial and there is no evidence of decay or mummification of badly diseased specimens, as there is with some gladiolus diseases. The diseased areas, which become very hard in texture, can be readily lifted out with the finger-nail, thus exposing the healthy tissue in a shallow, crater-like depression.

The symptoms on susceptible varieties are very similar except for a few minor differences. In the variety Duna only a few ring spots are found on a corm but they are of considerable diameter. The lesion very often extends twice as deeply as in Amrita and healthy tissue has never been observed between the concentric rings, although, in this variety, the rings are set off sharply in colour. The ring spots formed in the varieties Snowwhite, Barcarole, Honour, Roselle, and in some other seedlings manifest slightly different shades of a reddish-brown colour. In Barcarole and Honour there is a tendency for the rings of the lesion to be somewhat widely separated.

Development of the Disease in Naturally Infected Stock

Under Normal Storage Conditions

In the Vineland district, gladiolus corms are harvested about the middle of October and stored until early in May. Observations made during the 1940 storage season showed that in the variety Amrita, symptoms did not appear for two or two and one-half months. During the next two and one-half months, the number of new infections increased rapidly as did the size of spots already present. In the last third of the storage life only the occasional

PLATE I

FIG. 1. *Diseased Dickson corm. Several rings constitute a lesion, some of which are separated by healthy tissue.*

FIG. 2. *Diseased Dickson corm showing concentric rings in lesion separated by healthy tissue.*

FIG. 3. *Above: Diseased Amrita corm showing discoloration and loss of lustre in husks. Below: Healthy Amrita corm.*

FIG. 4. *Diseased Amrita corm showing very superficial ring spots completely covering the surface.*

new infection appeared while most of those already present continued to advance slowly. After seven months there was no further appreciable disease development. In general, the disease expression in most of the susceptible varieties followed this trend although in one or two, symptoms did not appear for three or more months.

Under Conditions of Modified Temperature and Humidity

Immediately after Harvesting

Some gladiolus growers "cure" corms by exposure to a high temperature for a few days after harvest to reduce the loss from storage diseases. To test the possible effect of temperature and humidity, corms from diseased stock of the varieties Amrita and Snowwhite were stored as follows: 10 corms at 60° F. for the whole period; 10 at 85° F. and normal humidity for 10 days; then at 60° F. for the remainder of the period; 10 at 85° F. and 100% relative humidity for 10 days, then at 60° F. for the remainder of the period. All the corms became badly diseased and it appeared that the different temperatures or humidities during the 10-day "curing" period had no appreciable effect on ring spot development.

Throughout Storage

The effect of various temperatures and humidities throughout the whole storage period was investigated. Corms of Amrita and of some Dickson seedlings grown from moderately diseased stock were stored at 58 and 94% relative humidity and 40° to 50° F. and 68° F.

After three months' storage there were fewer infections and slightly smaller ring spots on the corms stored at 40° to 50° F. At the end of six months, however, such ring spots were as large and extended slightly deeper into the tissues than those on corms stored at 68° F. Moreover, there was virtually no difference in the type of lesion obtained at the different relative humidities at the same temperature.

Relation Between Time of Harvest and Disease Development

Corms from diseased stock were dug at weekly intervals from Aug. 18 to Oct. 26 and stored in paper bags in the storage cellar. In each lot infections appeared in approximately two and one-half months after digging. The time at which the corms were harvested thus affected only the time at which the disease first appeared and had no effect upon the subsequent development of the disease.

Experimental Planting of Corms

Diseased Corms in Unsterilized Soil

Observation of plants grown in the garden and greenhouse showed that heavily diseased and healthy corms produced foliage and bloom comparable in time and quality. No additional ring spots developed on diseased corms after planting nor was there any further development of those already present or any premature decay of the corms as in some other gladiolus diseases (2).

To study the persistence of the disease, corms of the varieties Duna, Amrita, and Barcarole were divided into three groups on the basis of number and size of ring spots, i.e. heavily, medium, and lightly infected. The position and size of the ring spots on some of the lightly infected corms were accurately noted and the corms were planted in a sandy loam soil. After harvesting, and storage for six months, critical examination showed that the progeny of the diseased corms was almost invariably diseased to some extent, the incidence varying from corm to corm and from group to group. In general, the progeny tended to be more heavily infected than the parents although some heavily infected corms produced only moderately diseased ones. On the whole, the position of the ring spots on the parent corm bore no obvious relation to the position on the progeny.

Healthy Corms in Sterilized and Unsterilized Soil

To test soil, known to have grown diseased corms, as a source of inoculum, healthy corms of the variety Snowwhite were planted in pots of unsterilized soil, sterilized soil, and in various mixtures of each and placed outdoors in a protected cold frame for the summer. In late October, each group was harvested and stored for six months. A critical examination at that time showed that there was no significant difference in the percentage of diseased corms in any of the soils nor in the severity of the disease in the various groups (see Table I). Thus it appears that non-sterile soil was not a source of inoculum.

TABLE I

NUMBERS OF CORMS DISEASED WHEN HEALTHY STOCK WAS PLANTED IN VARIOUS MIXTURES OF STERILE AND NON-STERILE SOIL

Composition of soil	Number harvested	Number diseased
All sterilized	5	3
$\frac{7}{8}$ Sterile, $\frac{1}{8}$ non-sterile	5	2
$\frac{3}{4}$ Sterile, $\frac{1}{4}$ non-sterile	4	3
$\frac{5}{8}$ Sterile, $\frac{3}{8}$ non-sterile $\frac{1}{2}$ Sterile, $\frac{1}{2}$ non-sterile $\frac{3}{8}$ Sterile, $\frac{5}{8}$ non-sterile	* 14	11
$\frac{1}{4}$ Sterile, $\frac{3}{4}$ non-sterile	5	4
$\frac{1}{8}$ Sterile, $\frac{7}{8}$ non-sterile	5	3
All non-sterile	7	3

* *The corms from these pots were placed by mistake in one container at harvesting.*

Histological Studies

Microscopic examination of stained free-hand sections through diseased areas revealed that the lesion rarely extended deeper than 15 cells into the corm. The average depth of the lesion varies, however, to some extent in different varieties. In the variety Amrita the lesions tend to be shallow and usually do not involve more than eight or nine cells. In the varieties Duna and Snowwhite and in some other seedlings, the lesions often extend from 10 to 18 cells into the tissue of the corm, or approximately twice as deeply as in Amrita.

In incipient infections the epidermal cells and the parenchymatous cells in the first and second layer beneath the epidermis assume a brownish colour. These cells appear dead and stain uniformly with neutral red. The browning of the cells is progressive and is concomitant with the disappearance of starch from the cells in the infected region. In old lesions the cells assume a collapsed appearance and frequently adjacent to the nodes is located a group of brownish-coloured, collapsed cells which extend rather deeply into the corm tissue (Fig. 5).

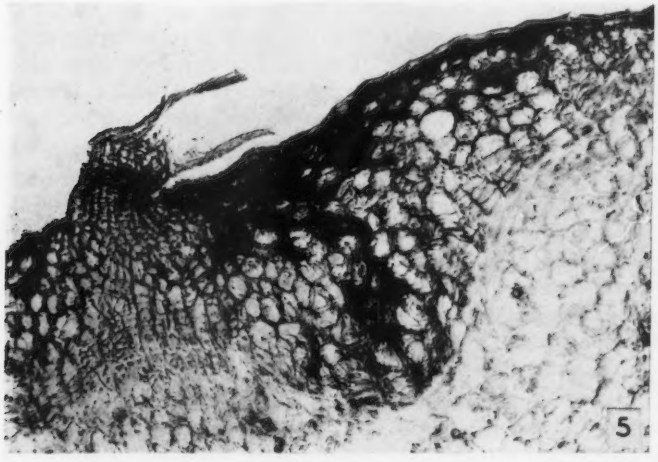

FIG. 5. *Section through a lesion of a diseased Amrita corm. Lesion attains greatest depth immediately adjacent to leaf base. Periderm layer separates diseased from healthy tissue.*

Infections in 90% of the cases begin at the node and in this region too the lesions attain the greatest depth. The lesion is separated from the healthy tissue of the corm by a periderm layer of 5 to 10 cells in thickness (Fig. 5). These cork cells are formed soon after the infection becomes apparent and may be in part responsible for the small dimensions and shallow depth attained by some of the lesions. When an infection consists of two or three definitely

separated concentric rings, the tissue between the rings appears normal, and each ring of infected tissue is walled off by a cork layer.

When stained free-hand sections of diseased corm tissue were examined under the microscope, occasionally fungus mycelium was found in deep lesions but never in small incipient infections. The mycelium in these ring spots was sparse and could have been easily overlooked because it stains very faintly and does not form any striking contrast with the background. After diseased corms were planted for three weeks in sterile sand, the fungus mycelium in the lesions was more plentiful and stained more deeply with cotton blue than when the corms were in storage.

From a comparison of susceptible varieties with King Lear and Camellia on which ring spots have never been found, resistance to ring spot does not appear to be due to any obvious morphological characteristics of corms. The cell size, number and character of the stomata over the nodal and internodal regions were much the same in all varieties.

Attempts to Isolate a Causal Organism

Repeated attempts were made throughout the summer of 1939 to isolate a pathogen from old ring spot lesions on corms that had been in storage from 7 to 10 months. Small slices of the ring spots were surface sterilized and planted on agar in Petri plates. In approximately 25% of the isolations, organisms appeared; from these a few *Penicillium* and a few *Fusarium* isolates were obtained, *Penicillium* appearing more frequently.

During the 1940 storage season, periodic isolations were made, from the time of appearance of initial infections until the corms had been in storage eight or nine months, ring spots of various diameters being used. The tissues were plated on potato dextrose agar, malt agar, gladiolus agar, and in nutrient broth. From incipient infections, a few cultures were obtained, one of *Fusarium* and a few of *Penicillium*, from the smallest ring spots. Once again a very large percentage of isolations did not yield any organisms and this was particularly true in isolations from incipient infections. Infrequently, however, from the smallest spots a *Penicillium* was obtained and a *Fusarium* isolate was secured only on one occasion out of hundreds of isolations. From the larger lesions, which appeared later in storage, in approximately 25% of the isolations organisms were obtained. The ratio of *Penicillium* to *Fusarium* isolated from the medium-sized lesions was about 10 to 1 and was much greater in the smaller lesions. From all of these isolations there were obtained five different isolates of *Penicillium* and three different isolates of *Fusarium*, as judged by differences in colonial characteristics on agar. In nutrient broth cultures occasionally one or two bacterial colonies developed from the bits of diseased tissue.

Since the use of a surface sterilizing agent introduces a complicating factor into any isolation technique, and particularly so in a superficial lesion of this sort, the following attempts were made to dispense with surface sterilization.

(*a*) Lesions were sliced from the corm and split across the centre by folding backwards, leaving only the epidermal surface of the lesion intact. Bits of diseased tissue were removed aseptically with the tip of a sterile scalpel from the lower part of the lesion along the split edge and transferred to the surface of an agar plate. Once again, in most of these isolations, no organisms were obtained, but on three occasions a *Penicillium* was isolated and once a *Fusarium* colony grew from the diseased tissue. Owing to the extremely superficial nature of initial infection centres, the small number of times in which organisms were isolated, and the ease with which contaminants entered, the procedure could not be employed as a routine method in attempted isolations.

(*b*) Isolations were also made after passing bits of diseased tissue through several transfers of sterile water, thus dispensing again with a surface sterilizing solution. Owing to the discouragingly large number of organisms that grew from the lesions, this method was discontinued.

From all of these attempts at isolation no single organism was obtained consistently. However, since a number of *Penicillium* and *Fusarium* spp. and occasionally bacteria were isolated, it seemed desirable to discover if these organisms were pathogenic and capable of producing the ring spot symptom.

Inoculation Experiments

Throughout the summer of 1939, corms were inoculated with one *Penicillium* and one *Fusarium* which had been isolated most frequently. Several half-inch square blocks of agar on which the fungus had been growing for one week were applied to the surface of healthy Amrita corms that had been in storage for 8 to 10 months. Examinations at fortnightly intervals for two months revealed no infection.

Because of the negative results obtained, two additional possibilities were investigated, viz., that the parasite might be a wound parasite or that the gladiolus corm might be susceptible only at certain precise stages in its ontogeny.

Beginning in early August, 1940, inoculations were conducted at fortnightly intervals until the end of February, 1941. Some corms were inoculated while growing in the field and greenhouse, the rest in the storage cellar, the procedures being as nearly identical as possible. Corms of the varieties Amrita and Snowwhite were inoculated with two *Fusarium* isolates, five of *Penicillium* and one *Bacterium*, separately and in combination. The organisms were applied on a half-inch square of cheesecloth supporting a block of agar on which it had been growing 10 days. Beneath this a wound was sometimes made through the leaf bases to the surface of the corm. No infections resulted from any of these inoculations.

In other corm inoculations, previously isolated fungus spores and bits of fungus mycelium and bacteria were suspended in a 2% dextrose solution and injected around the nodes between the leaf sheaths. After three-months'

incubation under normal storage conditions there was no evidence of infection. Additional inoculations, in which excised bits of diseased tissue were applied to the nodal areas of healthy Amrita corms, did not produce any infections.

Discussion

Under a wide range of temperature and moisture conditions in storage neither the time of appearance of ring spot symptoms nor the disease expression has been modified to any appreciable extent. Owing to the consistency of this unique symptom expression and the lack of leaf and flower symptoms, this trouble may be regarded as distinct from other common gladiolus diseases. If it is compared with these, it is found that the hard rot (*Septoria gladioli*) and dry rot (*Sclerotinia gladioli*) diseases are progressive in storage and may reduce infected specimens to mummies. In both Penicillium rot (*Penicillium gladioli*) and Fusarium rot (*Fusarium oxysporum* var. *gladioli*) concentric rings may be present in the lesion, but symptoms are modified to a great extent by temperature and moisture conditions in the early storage period and much rotting of the corm tissues is often evident. Fusarium yellows is a vascular disease with quite different symptoms. In contrast with all of these the ring spot disease displays neither field symptoms nor progressive rotting in storage. Characteristic scab (*Bacterium marginatum*) lesions have been observed on both the leaves and corms of varieties susceptible to ring spot and the causal bacterium is readily isolated from such lesions. The ring spot symptom resembles that of scab only in the superficial nature of the lesion on the corm. The bacteria occasionally isolated from ring spot lesions were not *Bacterium marginatum*.

The experiments which have been detailed unfortunately have not established the cause of the ring spot disease of gladiolus. They do not even permit one to say unequivocally whether the disease is caused by a parasite or whether it is non-parasitic in nature. Some of the possible etiological factors will now be considered.

The disease may be caused by a virus. This might perhaps be suggested by the ring spot symptom which in foliar tissues is characteristic of a group of viruses. Also the rather consistent development of this disease in progeny of affected corms in the absence of any demonstrable parasite suggests a virus origin. It would seem to be atypical of a virus, however, to express itself solely as a ring spot in a storage tissue. Furthermore, mechanical inoculations into healthy corms using sap expressed from diseased tissue and by inserting pulp of diseased tissue into healthy corms have yielded negative results, as have also preliminary experiments using thrips as possible vectors in storage.

The possibility of the disease being of insect origin appears to be remote. No evidence of insect punctures has been observed in histological studies. Thrip activity presents the only likely possibility, but characteristic disease symptoms have appeared on corms where thrips have been kept under control, and both ring spot and thrip injury have been observed on individual corms.

That the disease may be non-parasitic is suggested primarily by the apparent lack of any consistent association with any pathogen. It is difficult, however, to reconcile a non-parasitic physiologic disturbance with a ring spot symptom and to conceive of the cause being a physiologic one when frequently only a few corms of the same lot of one variety in a given storage container develop symptoms. Also, neither maturity of corms nor stagnation of air in storage has modified symptom expression, as is often characteristic of non-parasitic disorders.

The writer postulates that the disease may be caused by a soil fungus of a low order of aggressiveness that can attack the host only under conditions that pertain when corms have been stored for some time. It may be significant that the initial point of attack is at the base of the leaf sheaths, that is, in the region where a saprophytic development of the fungus would be possible for a considerable time in storage. The negative results of artificial inoculations might also be readily understood in the light of a combination of weak parasitic capabilities and susceptibility correlated with the development rhythm of the corms.

Attempted Control Measures

Although this ring spot disease does not damage the foliage or bloom of gladiolus it is highly undesirable in propagative stock. Owing to the prevalence of the disease in certain popular lines control measures were attempted. Treating diseased corms with formaldehyde, mercuric chloride, semesan, and calcium hypochlorite before planting in garden soil afforded no obvious control. A series of experiments in which gladiolus corms from diseased stock had been dipped in solutions of formaldehyde, mercuric chloride, semesan, and calcium hypochlorite of recommended strengths at harvest time also effected no control.

Since investigations on this problem have to be discontinued at least for the duration of the war it seems advisable to report on them at this time.

Acknowledgments

The writer wishes to express his sincere gratitude to Professor D. L. Bailey, at whose suggestion the problem was undertaken, for his constant help and encouragement during its investigation. Acknowledgments are also due Professor E. F. Palmer and Mr. G. H. Dickson of the Vineland Horticultural Experiment Station, for placing so much experimental gladiolus material at the writer's disposal.

References

1. HOPP, H. Control of atmospheric humidity in culture studies. Botan. Gaz. 98 (1) : 25-44. 1936.
2. MOORE, W. C. Diseases of bulbs. Ministry Agr. Fisheries (Engl.), Bull. 117. 1939.

ADULT PLANT RESISTANCE IN WHEAT TO PHYSIOLOGIC RACES OF *PUCCINIA TRITICINA* ERIKSS.[1]

By Margaret Newton[2] and T. Johnson[3]

Abstract

Nine wheat varieties were studied in two stages of growth, the seedling stage and the heading stage, for their reaction to a number of physiologic races of leaf rust of wheat, *Puccinia triticina* Erikss. Several varieties susceptible to certain physiologic races in the seedling stage were found to acquire, as they grew to maturity, a resistance to these races. In the adult plant, the resistance was greatest in the uppermost leaves but diminished progressively on lower leaves. The varieties tested fell into three groups on the basis of their rust reaction. (1) Renown and Regent, which developed adult plant resistance to all of the 19 races to which they were tested. In these two varieties, and possibly in other derivatives of H-44 and Hope, adult plant resistance to physiologic races of leaf rust may be a generalized phenomenon comparable to the resistance such varieties show towards stem rust. (2) Thatcher, Apex, Marquis, Reward, and Kenya R.L. 1373, which showed adult plant resistance only to certain physiologic races, a condition not hitherto encountered in other cereal rusts. (3) McMurachy and Warden × Hybrid, each of which reacted somewhat similarly in the seedling and heading stages, the former being susceptible and the latter resistant in both stages.

Introduction

The fact that wheat varieties susceptible to *Puccinia triticina* Erikss. in the seedling stage may develop resistance in later growth stages to this rust has been noted by several investigators. Mains and Jackson (6) observed that some wheats moderately susceptible as seedlings became highly resistant as they matured. Gassner (1) found, in field experiments conducted in South America, that certain wheat varieties susceptible in early stages of growth acquired more or less resistance in the jointing stage. This resistance, however, appeared to be transient in that it was frequently replaced in later developmental stages of the plants, especially after they had flowered, by more susceptible reactions. Johnston and Melchers (5) showed clearly that a number of varieties of wheat susceptible in the seedling stage to physiologic race 9 were highly resistant at heading to the same race. Other investigators who have observed adult plant resistance to leaf rust include Vohl (12), whose work is reviewed elsewhere in this paper, and Obermeister (10), who found that, in Russia, several winter wheats susceptible in the seedling stage to physiologic race 13 were resistant in the jointing and heading stages. Scheibe (11) and Gassner and Kirchhoff (2), on the other hand, did not find any evidence of adult plant resistance in the varieties studied by them.

Despite the negative results obtained by Scheibe and by Gassner and Kirchhoff, it seems evident that adult plant resistance to races of leaf rust is

[1] *Manuscript received July 30, 1942.*

Contribution No. 718, Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

[2] *Senior Plant Pathologist, Dominion Laboratory of Plant Pathology, Winnipeg, Man.*

[3] *Plant Pathologist, Dominion Laboratory of Plant Pathology, Winnipeg, Man.*

characteristic of a number of wheats. The practical importance of this type of resistance, however, has not been made clear. It can obviously have little practical value unless varieties exist in which adult plant resistance to many physiologic races is developed. The absence of definite information on the relative reactions to leaf rust of seedling and adult plants in even the most commonly grown wheat varieties in Canada was one of the main reasons for undertaking the work described in the present paper.

Method of Estimating Rust Reaction

Throughout the experiments reported in this paper the same methods of determining rust reaction were used. When the reaction of adult plants was recorded, separate readings were taken on each rusted leaf of each plant examined, from the flag leaf downwards. The infection types present were noted and later converted into numerical values on the basis of 5 for type 1 infection, 10 for type 2, 15 for type 3, 20 for type 4, and 11, 12, 13, 14, and 15 respectively for types x=, x−, x, x+, and x++. The reactions given for adult plants in the various tables are based on averages for the four uppermost leaves, i.e., the flag leaf and the three leaves immediately below it. Observations on rust reaction were generally taken about 14 days after inoculation.

Seedling and Adult Plant Reactions of Wheat Varieties in the Greenhouse

With leaf rust, as with other cereal rusts, tests for rust reaction are often carried out on plants in the seedling stage to gain some idea of the resistance or susceptibility of a variety to a given physiologic race. That the reaction to leaf rust of plants in the seedling and the adult stage may differ considerably will be clear from an examination of Table I, which compares the reaction to seven physiologic races of eight wheat varieties in the seedling and the heading stage. Only one of the eight wheats, namely, the variety McMurachy, proved equally susceptible in both growth stages to all of the physiologic races. Five varieties (Thatcher, Apex, Marquis, Kenya, Reward) that as seedlings were susceptible to all these races showed some resistance in the heading stage to one or more races. The H-44 derivatives, Renown and Regent, which are generally regarded as resistant to leaf rust in the field (7), proved to be in a class by themselves in that they were resistant or moderately resistant in the heading stage to all races. In Renown, but not in Regent, resistance was present also in the seedling stage though, to most races, it was less pronounced than at heading. The seedling tests agreed with previous tests (9) in which Regent was found to be susceptible or moderately susceptible to eight physiologic races whereas Renown was for the most part moderately resistant.

The resistance or moderate resistance of Renown and Regent in the adult stage to the seven physiologic races in this test suggested the possibility that these varieties might possess a general resistance to the leaf rust races prevalent

TABLE I

A COMPARISON OF SEEDLING AND ADULT PLANT REACTION OF EIGHT WHEAT VARIETIES TO SEVEN PHYSIOLOGIC RACES OF *Puccinia triticina* IN THE GREENHOUSE

Phys. race	Stage of growth	Thatcher	Apex	Marquis	Regent	Renown	McMurachy	Kenya R.L. 1373	Reward
1	Seedling	S	S	S	MS	MR	S	S	S
	Adult	MS	MR	MS	R	R	S	S	S
2	Seedling	S	S	S	MS	MR	S	S	S
	Adult	S	MS	S	MR	MR	S	MR	S
15	Seedling	S	S	S	MS	MR	S	S	S
	Adult	MS	MS	MS	R	R	S	MS	S
27	Seedling	S	S	S	MS	MR	S	S	S
	Adult	MR	MR	MR	R	R	S	MR	MR
28	Seedling	S	S	S	S	MR	S	S	S
	Adult	S	S	MS	MR	MR	S	S	S
29	Seedling	S	S	S	S	MR	S	S	S
	Adult	MS	MS	MS	MR	R	S	S	S
71	Seedling	S	S	S	S	MR	S	S	S
	Adult	MS	MS	MS	MR	MR	S	S	S

NOTE: S = *susceptible;* MS = *moderately susceptible;* MR = *moderately resistant;* R = *resistant.*

in North America. To gain further information along this line, Renown and Regent were inoculated at the time of heading with 12 additional races, namely, races 3, 20, 31, 34, 39, 44, 52, 58, 83, 89, 104, and 130. Both varieties proved resistant or moderately resistant to all these races. It therefore seems probable that these two wheats exhibit towards North American races of leaf rust a resistance as general though not so great as the resistance they show to physiologic races of *Puccinia graminis Tritici* Erikss. and Henn.

It is apparent from the data presented in Table I that, in contrast to the general leaf rust resistance of adult plants of Renown and Regent, some wheats may show an adult plant resistance specific to certain physiologic races. Thus Thatcher, which, on the basis of behaviour in the field, is regarded as susceptible to leaf rust, proved nevertheless resistant in the adult stage to race 27. A further test of the reaction of Thatcher in seedling and heading stages to 10 physiologic races (Table II) showed it to possess adult plant resistance to races 9 and 31 as well, although it proved susceptible in both these stages of development to the other eight races used in the test.

It may well be that adult plant resistance specific against certain leaf rust races is characteristic of many wheats. Adult plant resistance to certain races was shown by five of the eight varieties whose reactions are recorded in Table I. In the field, this kind of resistance may not be of much importance owing to the fact that leaf rust epiphytotics are usually caused by many physiologic races. Resistance against one or two races may therefore be masked by severe infection produced by other races.

TABLE II

REACTION OF THATCHER WHEAT IN THE SEEDLING STAGE AND IN THE HEADING STAGE TO 10 PHYSIOLOGIC RACES OF LEAF RUST IN THE GREENHOUSE

Experiment number	Time of test	Physiologic race	Seedling reaction	Adult plant reaction			
				Flag leaf	2nd leaf	3rd leaf	4th leaf
1	Jan. 1940	9	S	I	I	R	MR
		76	S	S	S	MS	MS
2	Feb. 1940	9	S	R	R	R	MR
		71	S	S	MS	MS	MS
3	May 1940	9	S	MR	MR	MR	MR
		15	S	S	S	S	S
		31	S	MR	MR	MR	MR
		41	S	S	S	S	S
		52	S	S	S	S	S
		53	S	S	S	S	S
		71	S	S	S	S	S
		76	S	S	S	S	S
		89	S	S	S	S	S
		103	S	S	S	S	S

NOTE: S = *susceptible;* MS = *moderately susceptible;* MR = *moderately resistant;* R = *resistant;* I = *immune.*

It should be mentioned that varieties exhibiting adult plant resistance to a given physiologic race do not usually show a uniform resistance throughout the whole plant. The highest resistance is almost always shown by the uppermost or flag leaf, the second, third, and fourth leaves showing a progressively diminishing resistance (Table II). This behaviour, which was noted by Johnston and Melchers (5) and by Vohl (12), appears to be characteristic of wheat varieties possessing adult plant resistance.

Seedling and Adult Plant Reactions of Wheat Varieties in the Field

Experiments on the reaction of wheat varieties grown in the field to individual physiologic races of leaf rust have been conducted by Gassner and Kirchhoff (2), Scheibe (11), and Vohl (12). Gassner and Kirchhoff's experiments, which did not include the reaction of plants prior to the jointing stage, led to the conclusion that the susceptibility to race 14 of leaves attached about the middle of the stem increased until the time of flowering, while the susceptibility of the flag leaf reached a maximum somewhat later. Scheibe grew his wheat varieties in the field and transplanted them to the greenhouse where they were inoculated with races 11, 13, 14, and 15. He concluded that varieties highly resistant or completely susceptible in the seedling stage, remained so at all growth stages, but that varieties showing a moderate resistance as seedlings showed still greater resistance in more advanced stages of growth. Vohl, for two successive years, grew 15 wheat varieties in the

field where he inoculated them with the four physiologic races used by Scheibe. He found that 14 of the 15 varieties studied became resistant as adult plants to races to which they were susceptible as seedlings. The varieties with adult plant resistance to all four of the races used included Thatcher, Reward, Garnet, and Marquis, which, in Canada, are definitely susceptible to leaf rust under field conditions.

The differences in the results obtained and, therefore, in the conclusions arrived at by Scheibe and Vohl may be due to the fact that they worked with different wheat varieties; or they may be attributable to differences in experimental methods, as Scheibe grew his varieties in the field and transplanted them to the greenhouse, whereas Vohl made his observations on plants grown entirely in the field.

With a view to determining whether or not the reaction of a variety in the field differed from its reaction in the greenhouse, field experiments were conducted in Winnipeg in the summers of 1940 and 1941. The success of field experiments with specific physiologic races depends on the absence during the experimental period of any appreciable amount of natural inoculum of the rust under study. This condition was realized in 1940 but not in 1941.

In 1940, five varieties, Warden × Hybrid, Renown, Regent, Thatcher, and McMurachy, were sown at three different dates, April 26, May 17, and June 7, in four plots separated by wide buffer plots of oats. Inoculations were made on June 25 with races 5, 9, 71, and 76, each race being used to inoculate a single plot comprising all five wheat varieties. At that time, plants of the first sowing were in shot blade, those of the second sowing in the early jointing stage with a height of about 10 in., while those of the third sowing were in the two to three leaf stage. Epidemiological records of the prevalence of natural inoculum showed that by June 25, when inoculations were made, 13 wind-borne spores of leaf rust per square inch had fallen at Winnipeg and that by June 30 a total of only 16 spores had fallen. When, on July 11, notes were taken on rust infection only a trace of natural infection was found on adjacent susceptible wheats.

A similar experiment had to be discarded in 1941 owing to the early appearance of naturally occurring inoculum. In that year inoculations were made on June 23. Epidemiological records showed that by that date wind-borne spores of leaf rust to the number of 571 per square inch had fallen at Winnipeg and that by June 30 the number had mounted to 2837. Infection from this wind-borne inoculum was so great as to render uncertain the reaction of the varieties to the races used as inoculum.

The results of the 1940 experiment are summarized in Table III. The reaction of each variety at each growth stage was arrived at as a result of five separate readings of the pustule types present on the leaves. Where comparison with greenhouse tests is possible it appears that the results obtained in the field are not markedly different from those secured in the greenhouse. Thatcher proved to have at least a moderate adult plant resist-

TABLE III

REACTION IN THE FIELD OF FIVE WHEAT VARIETIES AT THREE DIFFERENT GROWTH STAGES TO FOUR PHYSIOLOGIC RACES OF *Puccinia triticina*

Variety and stage of growth	Race 5	Race 9	Race 71	Race 76
Warden × Hybrid[1]				
2–3 leaf stage	I	I	R	R
Jointing stage	I	I	I	R
Boot stage	I	I	I	R
Renown				
2–3 leaf stage	MR	MS	MS	MS
Jointing stage	R	R	MR	R
Boot stage	R	R	R	R
Regent				
2–3 leaf stage	MR	MS	S	MS
Jointing stage	R	MR	MR	R
Boot stage	R	R	R	R
Thatcher				
2–3 leaf stage	S	MS	S	MS
Jointing stage	S	R	S	S
Boot stage	S	MR	S	MS
McMurachy				
2–3 leaf stage	S	S	S	S
Jointing stage	MS	MS	S	MS
Boot stage	MS	MR	MS	MS

NOTE: S = *susceptible;* MS = *moderately susceptible;* MR = *moderately resistant;* R = *resistant;* I = *immune.*

[1] *Received by Dr. R. F. Peterson of the Dominion Rust Research Laboratory, Winnipeg, from Dr. R. M. Caldwell, Purdue University Agricultural Experimental Station, under the designation Warden C.I. 4994–4x Hybrid English W. 325.*

ance to race 9 but none to races 71 and 76, a result in agreement with that obtained in the greenhouse. Renown and Regent, which in the greenhouse showed adult plant resistance to all races to which they were tested, showed a similar resistance to the four races used in the field. McMurachy, however, which in the greenhouse was susceptible in both seedling and adult stage, appeared in the field to have a slight amount of adult resistance to one of the four races. Warden × Hybrid, which has not been tested in the adult stage in the greenhouse, proved immune or highly resistant in the field to the four races employed.

It is not possible to make a direct comparison of this experiment with the above-mentioned field experiments conducted by Vohl (12), in Germany, because the same races were not used and only one variety, Thatcher, was common to both experiments. The reaction of this variety is so strikingly different in the experiments recorded here and in those reported by him as to call for some comment. In his experiments, Thatcher showed high adult plant resistance for two successive years to races 11, 13, 14, and 15, all of which have been collected in both Canada and the United States. In greenhouse and

field tests at Winnipeg Thatcher was susceptible in the seedling and adult stages to all races to which it was tested, except the three somewhat similar races 9, 27, and 31. Moreover, there is abundant evidence that, in North America, Thatcher is highly susceptible to leaf rust; and has, in fact, suffered severe reduction in yield from leaf rust infection in certain years, notably 1938 and 1941. It is remarkable, therefore, that it should have shown adult plant resistance to the four European races to which it was tested. Presumably the Thatcher used by Vohl was identical with that distributed in the United States in 1934, as Vohl's work was done in 1935 and 1936. It is improbable, therefore, that differences in genetic constitution between the material used by Vohl and that used in the present experiment could account for the differences between his results and those presented in this paper. One possible explanation of Vohl's results would be that the races of leaf rust used by him differ pathogenically from the American races designated by the same race numbers. Some support is lent to that supposition by the fact that Thatcher, which, according to Vohl, has adult plant resistance to race 15 was completely susceptible in both seedling and adult stages to a Canadian collection of race 15 (Table II). There is nothing inherently impossible in such an explanation, for it has been shown by Waterhouse (13) and by Waterhouse and Watson (14) that the Australian variety Thew may react quite differently to two cultures of leaf rust that cannot be distinguished on the standard differential hosts for that rust. Another possible explanation may be sought in Vohl's experimental methods. Successive leaves on his plants were inoculated as they emerged so that, for example, in 1936, the first three or four leaves were inoculated on April 22, whereas the leaves at the heading stage were inoculated on June 18. It is therefore possible that the greater resistance of the leaves at later growth stages of varieties such as Thatcher, Garnet, and Reward may have been induced by a progressive rise in temperature. Although it has been shown (3, 4, 8) that certain wheat varieties are more resistant at high than at low temperatures, the plausibility of this explanation of Vohl's results is lessened by the fact that a somewhat similar seasonal rise in temperature, in North America, does not appear to induce resistance to leaf rust in the varieties mentioned above.

It would appear from the experimental results reported in this paper that seedling reactions are by no means reliable indices to the leaf rust reactions of adult plants, at least when the seedling reaction is of a susceptible type. On the other hand, the experience of the writers indicates that, when the seedling reaction is of a resistant type, it is a satisfactory guide to the reaction of the adult plant. It is evident that many wheat varieties that are susceptible in the seedling stage become progressively less susceptible as they mature. Adult plant resistance is usually, perhaps always, greatest in the uppermost (flag) leaf and diminishes progressively downwards in the leaves below. Certain varieties appear to develop adult plant resistance to most or all of the physiologic races of leaf rust prevalent in Canada. Of the varieties tested, only Renown and Regent showed this generalized resistance which is likely

derived from their H-44 parent and is probably shared by Hope and some of its derivatives. It may be remarked that these varieties possess a similar generalized adult plant resistance to physiologic races of wheat stem rust. Certain other varieties, such as Thatcher and Marquis, develop an adult plant resistance specific to a few physiologic races. Resistance of this type is not likely to be of much practical value unless the particular races against which it is operative should happen to predominate in a natural epidemic. Adult plant resistance to specific races has apparently not been reported for other cereal rusts.

References

1. GASSNER, G. Phytopath. Z. 4(6) : 549-596. 1932.
2. GASSNER, G. and KIRCHHOFF, H. Phytopath. Z. 7(1) : 43-52. 1934.
3. GASSNER, G. and STRAIB, W. Phytopath. Z. 4 (1) : 57-64. 1931.
4. JOHNSON, T. and NEWTON, M. Can. J. Research, C, 15 (9) : 425-432. 1937.
5. JOHNSTON, C. O. and MELCHERS, L. E. J. Agr. Research, 38(3) : 147-157. 1929.
6. MAINS, E. B. and JACKSON, H. S. Abstract. Phytopathology, 11(1) : 40. 1921.
7. NEWMAN, L. H., FRASER, J. G. C., and WHITESIDE, A. G. O. Can. Dept. Agr. Pub. 538. Farmers' Bull. 18. (Revision.) 1939.
8. NEWTON, M. and JOHNSON, T. Can. J. Research, C, 19 (4) : 121-133. 1941.
9. NEWTON, M., JOHNSON, T., and PETURSON, B. Can. J. Research, C, 18(10) : 489-506. 1940.
10. OBERMEISTER, N. P. Trudy Zashchite Rastenii (Bull. Plant Protection U.S.S.R.), No. 1–2 : 222-227. 1940.
11. SCHEIBE, A. Arb. biol. Reichs. Land-u. Forstw. Berlin-Dahlem, 17(6) : 549-586. 1930.
12. VOHL, G. J. Z. Zücht., Riehe A, Pflanzenzücht. 22(2) : 233-270. 1938.
13. WATERHOUSE, W. L. Proc. Linnean Soc. N.S. Wales, 54 (5) : 615-680. 1929.
14. WATERHOUSE, W. L. and WATSON, I. A. Proc. Linnean Soc. N.S. Wales, 66 (5 and 6) : 269-275. 1941.

STUDIES ON FOOT AND ROOT ROT OF WHEAT

VII. SOME FACTORS AFFECTING THE HEALTH OF WHEAT SEEDLINGS IN NUTRIENT SOLUTIONS[1]

By L. E. Tyner[2] and W. C. Broadfoot[3]

Abstract

The effect of iron tartrate on the development of chlorosis in wheat seedlings in nutrient solutions, and also the effect of extracts of *Helminthosporium sativum* P. K. & B. and *Fusarium culmorum* W. G. Sm. on disease expression, were studied under greenhouse conditions. Iron tartrate was effective in preventing chlorosis. Less iron was required in summer plantings than in winter plantings. Also, less iron was required in solutions with the higher hydrogen ion concentrations. Under the conditions employed, manganese effected no amelioration of chlorotic symptoms in the presence of a deficiency of iron.

Sterilized and unsterilized filtered extracts of the pathogens mentioned added to crocks of the nutrient solution inhibited the growth of wheat seedlings, an effect in soil culture which is interpreted as an index of pathogenicity.

Introduction

Preliminary experiments (1) previously reported suggested that several factors involved in the technique of culturing wheat seedlings in nutrient solutions should be studied. The occurrence of chlorosis of the seedlings under certain conditions, the effect of pH of the solution upon the growth of the plant, and the effects of sugar and fungous staling products on the seedlings are to be considered in this paper. The first factor concerns iron requirements and utilization by the plant. The effect of fungous extracts is important because of its relation to the criteria usually employed in estimating pathogenic effects.

Materials and Methods

The experiments were carried out in the greenhouse. The complete nutrient solution used was made up according to a formula kindly forwarded by Professor D. R. Hoagland of the University of California and made up of molar solutions of C.P. calcium nitrate, potassium nitrate, magnesium sulphate, and monobasic potassium phosphate in the amounts of 5, 5, 2, and 1 ml. per litre of water, respectively. In addition, Hoagland's A–Z solution, supplying trace elements, was added in most plantings at the rate of 1 ml. per litre. This solution was made up by adding the following chemicals to 18 litres of distilled water: potassium iodide, potassium bromide, stannous chloride dihydrate, lithium chloride, 0.5 gm. of each; aluminium sulphate, titanium oxide, zinc sulphate, copper sulphate pentahydrate, nickel sulphate hexahydrate, cobalt nitrate hexahydrate, 1.0 gm. of each; manganese chloride

[1] *Manuscript received September 26, 1942.*

Contribution No. 724 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa.

[2] *Junior Plant Pathologist, Dominion Laboratory of Plant Pathology, Edmonton, Alta.*

[3] *Plant Pathologist, Dominion Laboratory of Plant Pathology, Edmonton, Alta.*

tetrahydrate, 7.0 gm.; boric acid, 11 gm. Infection of the seedlings was secured by immersing the roots in aqueous suspensions of spores and mycelium of *Helminthosporium sativum* P. K. & B. and *Fusarium culmorum* W. G. Sm.

Glazed, 1-gal. crocks were employed as containers of the nutrient solutions. The lids of the crocks were coated with asphalt paint before each planting. The cork rings used as holders for the seedlings were sterilized before each planting. Steam sterilization was usually employed, but later tests showed that immersion for 48 hr. in a solution of 1:1000 mercuric chloride with 10% methyl hydrate was effective and at the same time less injurious to the corks. For winter plantings daylight was supplemented by artificial light from 500-watt lamps for seven hours daily. Other details of methods employed will be described more appropriately under the individual plantings. The data were analysed by Fisher's (2) Analysis of Variance method and by the *F* test of Snedecor (5) to determine the significance of the differences observed among the various treatments.

Experiments and Results

Experiment VII

Effect of Concentration of Ferric Tartrate

In earlier experiments (1), iron, as ferric tartrate, in the proportion of 1 ml. of a 0.5% solution per litre of nutrient solution was added once a week, but in this planting it was added daily and in various concentrations. The concentrations were 0, 0.01, 0.03, 0.05, 0.1, 0.3, and 0.5%, which were added at the rate of 1 ml. per litre. The inoculum consisted of an aqueous suspension of *H. sativum*. After six days in contact with the pathogen, five apparently uniformly diseased seedlings were transplanted to crocks. Control plants were also transplanted. Replication was in quadruplicate. The experiment extended from December 2 to 27. The results are presented in Table I.

The development of the disease was satisfactory throughout. Length of shoot, and also green and dry weight, increased with an increase in the concentration of ferric tartrate. The increases in growth with increases of iron were greater in the control series than in those inoculated. In both series the greatest growth occurred in the solutions to which 1 ml. per day of 0.5% ferric tartrate was added to each litre of solution. The maximum positive growth response to iron was apparently not covered in the range of concentrations of ferric tartrate employed. However, it must be recalled that this test was made during winter, when chlorophyll development is weak. The addition of 1 ml. per litre of 0.5% ferric tartrate to the solutions thrice weekly has proved a satisfactory supplement for year-round plantings.

For these seedlings the minimum application of iron to the nutrient solution necessary to eliminate chlorosis was 1 ml. per litre per day of 0.1% ferric tartrate solution. It must be emphasized that this minimum applies only to this particular test. Subsequent experiments showed that under certain other conditions, e.g., when the chemical composition or the reaction of the nutrient

TABLE I

EFFECT OF DIFFERENT CONCENTRATIONS OF FERRIC TARTRATE IN THE NUTRIENT SOLUTIONS UPON THE GROWTH OF WHEAT SEEDLINGS PREVIOUSLY INOCULATED WITH *Helminthosporium sativum*

Ferric tartrate,† %	Average height, cm.		Average dry weight, mg.		Chlorosis, %	
	Inoculated	Control	Inoculated	Control	Inoculated	Control
0	26.8	28.4	80	100	90	95
0.01	27.3	30.0	76	100	50	60
0.03	29.7	32.8	72	102	20	25
0.05	29.3	30.7	82	102	10	15
0.1	32.2	34.7	90	142	0	Tr.
0.3	34.9	34.9	126	170	0	0
0.5	36.5	38.6	132	196	0	0

F value for	Height	Dry weight
Inoculation	11.00*	257.50*
Iron concentration	17.99*	95.25*
Necessary difference	3.4 cm.	31 mg.

† *Added to solution at rate of 1 ml. per day.*
* *Exceeds 1% point.*

solutions was altered, chlorosis was troublesome, even when the iron addition considerably exceeded the above-mentioned minimum.

As substantially the same results were obtained in a repetition of this planting using *F. culmorum*, there is nothing further to be gained by tabulating the results secured.

Experiment VIII

Effect of Applying Ferric Tartrate Daily and Weekly to Wheat Seedlings in Nutrient Solutions at Different pH Levels

Ferric tartrate solutions of 0.125, 0.25, and 0.5% concentrations were supplied to three crocks at the rate of 1 ml. per litre daily, and weekly in equivalent total amounts to another three crocks. Hoagland's complete nutrient solution was used throughout, and in three of the crocks it was adjusted to pH 5.5 with bi-weekly additions of normal C.P. sulphuric acid. Aqueous cultures of *H. sativum* and *F. culmorum* were used as inocula and the roots of the seedlings were in contact with these cultures for a period of six days before transplanting to the crocks where they were grown for 34 days (May 12 to June 15). The data are presented in Table II.

In the series to which no iron was added there was much more growth in the solutions kept at an acid reaction than in those to which no acid was added. The plants in the latter were much more chlorotic than those in the crocks to which acid was added. It would appear that, at a high hydrogen

TABLE II

EFFECT OF DIFFERENT CONCENTRATIONS OF FERRIC TARTRATE, APPLIED DAILY AND WEEKLY TO COMPLETE NUTRIENT SOLUTIONS AT TWO pH LEVELS, ON WHEAT SEEDLINGS PREVIOUSLY INOCULATED WITH *Helminthosporium sativum* OR *Fusarium culmorum*

Ferric tartrate, %	Average height, cm.			Average green weight, gm.		
	H. sativum	*F. culmorum*	Control	*H. sativum*	*F. culmorum*	Control
0 – *N*[1]	22.3	23.3	25.3	1.14	0.80	1.04
A[2]	35.3	31.8	36	5.52	3.24	6.66
Daily						
0.125 – *N*	52.7	50.7	63	7.72	5.74	11.14
A	53.8	48.8	64.2	7.90	4.52	12.00
0.25 – *N*	49.7	46.7	61.7	5.10	3.60	10.62
A	50.2	48.3	61.5	4.88	4.00	11.12
0.5 – *N*	55	48.3	57.3	5.02	3.90	8.00
A	56	47.8	60.3	6.02	3.70	8.88
Weekly						
0.125 – *N*	53.3	45.3	62	6.02	4.00	9.56
A	53.3	48.3	61.3	4.70	5.58	10.20
0.25 – *N*	54.3	53.7	63	6.06	5.88	12.20
A	55.3	50.2	64.8	5.78	4.58	13.00
0.5 – *N*	61.3	36.7	67.3	8.12	5.94	14.10
A	58.3	46.2	65.2	7.24	6.22	13.40

F value for	Height	Green weight
Inoculation	36.07*	146.93*
Iron concentration	22.72*	27.67*
Acidity	11.08*	4.84**
Necessary difference	11.6 cm.	2.6 gm.

* *Exceeds 1% point.*

** *Exceeds 5% point.*

[1] *N = approximately neutral reaction.*

[2] *A = adjusted to pH 5.5 twice weekly.*

ion concentration, traces of iron or possibly some substitute are made available, which at a lower hydrogen ion concentration cannot be utilized.

The relative effects of adding iron in large amounts once a week, or in small amounts daily, are noteworthy. In the crocks receiving weekly applications, the growth of the seedlings increased with increases in the amounts of iron supplied. Precisely the opposite reaction occurred in the crocks receiving the iron daily. The difference in response to iron from that in Experiment VII is thought to be a result of seasonal light conditions. In addition to the limit of tolerance shown to the dose of iron, there was also an apparent tendency for the iron supplied weekly to become unavailable, since the plants in these treatments were slightly chlorotic. All the inoculated plants were

less vigorous than those in the corresponding controls. The effect of inoculation was especially evident in plants inoculated with *F. culmorum*. The amount of disease, as expressed by retardation of growth, was not significantly different at pH values 5.5 and approximately 7.0 of the solutions.

Experiment IX

Effect of Manganese Added to Nutrient Solutions on the Utilization of Iron by Wheat Seedlings.

It has been stated by Shive (4) in a recent publication that manganese plays a very important role in the use of iron by the plant. The oxidation of the ferrous to the ferric form was said to be governed by the relative number of manganic ions present. He found that an imbalance of the ratio of manganese to iron present caused iron toxicity symptoms or deficiency symptoms according to the establishment of low manganese–high iron, or high manganese–low iron ratio, respectively. A low supply of iron was found to be satisfactory when manganese was also low.

Wheat seedlings were planted in 54 1-gal. crocks of complete nutrient solution lacking the A–Z solution. Eighteen of the crocks received 1 ml. of 0.5% ferric tartrate solution per litre thrice weekly, another 18 received 0.25 ml., and the remaining crocks received no iron. Each group of 18 crocks was further subdivided into three series of six crocks receiving initially 4, 1, and 0 p.p.m. of manganese supplied as manganese chloride. Half of all the treatments were brought to required volume with distilled water, and the other half with tap water. The plants were grown for five weeks during December and January. The results are given in Table III.

The type of water used for the solutions had no influence on growth. The growth increased with increases in the amount of iron added, but decreased as the amount of manganese in the solutions increased. Conditions were favourable for the development of chlorosis, which showed a regular increase with decrease in iron. However, it tended to average less in the series receiving no manganese.

From these results it is impossible to attribute any importance to the role of manganese in helping to control chlorosis in wheat seedlings as grown in the nutrient solutions employed.

Experiment X

Effect of Adding Aqueous Filtrates of H. sativum and F. culmorum to Nutrient Solutions in Which Wheat Seedlings were Grown

H. sativum and *F. culmorum* were grown separately in a sterilized, complete nutrient solution with 0.5% sucrose added. The fungi were cultured for 30 days in making the extracts used in Planting 1, and 70 days in Planting 2. The extracts were filtered free of spores and mycelium by passage through a Seitz-type filter. Both steam sterilized and unsterilized extracts were used in the first planting, but only unsterilized extracts in the second. These filtrates were then added in amounts of 25, 50, 100, 200, and 400 ml. to the

TABLE III

EFFECT OF VARIOUS CONCENTRATIONS OF MANGANESE AND IRON ON THE GROWTH OF WHEAT SEEDLINGS GROWN IN A COMPLETE NUTRIENT SOLUTION WITH DISTILLED AND TAP WATER

Manganese, p.p.m.	0.5% Ferric tartrate, ml.	Average height, cm.	Average dry weight, mg.	Chlorosis, %
Tap water				
4	1.0	33.3	140	20
	0.25	37	142	40
	0	32.7	84	90
1	1.0	42.7	216	10
	0.25	38.7	168	20
	0	33.3	82	90
0	1.0	45.3	252	0
	0.25	39.7	178	10
	0	31.7	96	90
Distilled water				
4	1.0	35.7	172	20
	0.25	33.7	130	40
	0	31.7	108	70
1	1.0	39.7	230	10
	0.25	40	186	30
	0	33.3	104	70
0	1.0	37.7	152	5
	0.25	39.7	200	10
	0	31.3	96	70

F value for	Height	Dry weight
Manganese concentration	13.44*	10.56*
Iron concentration	37.66*	71.18*
Water type	3.58	0.00
Necessary difference	4.1 cm.	44 mg.

* *Exceeds 1% point.*

1-gal. crocks of complete nutrient solution made up in distilled water. The controls in the first planting constituted crocks to which 25, 50, 100, 200, and 400 ml. quantities of a sterile solution of 0.5% sucrose were added, and also a comparable untreated series of crocks. The controls in the second planting were untreated. All treatments were replicated six times, and the crocks were completely randomized. The plantings were made in two successive seasons and were grown for 40 days during August and September. The data are presented in Table IV.

Contrasting the data on plant height with those on plant weight, it will be noted that the former criterion is much less sensitive to the effects of the treatments than the latter. Rather wide differences in weight are usually apparent

TABLE IV

EFFECT OF STERILIZED AND UNSTERILIZED TISSUE-FREE EXTRACTS OF *Helminthosporium sativum* AND *Fusarium culmorum* ADDED TO COMPLETE NUTRIENT SOLUTION CONTAINING WHEAT SEEDLINGS

Extract per crock, ml.	Average height, cm.			Average dry weight, gm.		
	Planting 1[1]		Planting 2[2]	Planting 1		Planting 2
	Sterilized	Unsterilized	Unsterilized	Sterilized	Unsterilized	Unsterilized
H. sativum						
25	43	45.2	50.8	0.65	0.82	0.89
50	44.8	44	49.7	0.76	0.64	0.79
100	43	41	46.3	0.49	0.46	0.67
200	42	42	48.3	0.55	0.44	0.63
400	45.5	41.3	48.5	0.60	0.41	0.67
F. culmorum						
25	44.3	41.7	53.0	0.70	0.78	1.05
50	44.8	41.8	51.8	0.69	0.69	0.90
100	42.7	45.2	51.2	0.64	0.70	0.92
200	47.2	42.2	47.8	0.74	0.55	0.78
400	43.5	41.2	46.0	0.62	0.48	0.65
0.5% Sucrose solution						
25	41.7			0.62		
50	40.8			0.61		
100	42.7			0.56		
200	38.8			0.45		
400	36.3			0.25		
Controls	44.0		52.0	0.84		0.93

[1] *Organism cultured for 30 days before making extracts.*
[2] *Organism cultured for 70 days before making extracts.*

before significant differences in height are recorded. Considering only the observed dry weight per plant, growth in the control units, containing nutrient solution only, exceeded that in all other treatments. Evidently the addition of 25 ml. of fungous filtrate to a gallon of nutrient solution was sufficient to retard growth to some extent. Larger amounts, particularly of unsterilized extracts, produced correspondingly greater effects. The reduction in growth with increasing amounts of the unsterilized extracts closely paralleled the results in those controls to which sucrose was added. The explanation of the differences in growth of seedlings with these two types of extracts must lie in the effects of sterilization on the by-products present in the fungal culture, because the unsterilized and sterilized extracts contain the same quantities of residual sugar. Repeated chemical tests by the iodimetric method of sugar determination of Shaffer and Hartmann (3) on extracts

of *H. sativum* and *F. culmorum* in these and other plantings have demonstrated that *F. culmorum* uses up all the added sugar during 30 days' growth. The observed data demonstrate that in the extracts of this organism there are at least two factors present that may cause reduction in growth of the wheat seedlings. One of these is heat labile and the other is not. *H. sativum*, on the other hand, did not use up all the sugar in its medium within 30 days, or even 70 days, and it must be assumed that at least part of the effects on growth observed by additions of extracts can be attributed to residual sugar.

Discussion

In earlier work (1), the occasional occurrence of chlorotic plants seemed likely to seriously interfere with the usefulness of the culture solution method of studying the root rots of cereals. In the various experiments just reported the conditions for adequate supply of iron received special attention; also the effect of extracts of the pathogen on plant growth and disease development was observed.

The addition of ferric tartrate solution at the rate of 1 ml. of 0.5% strength to each litre of nutrient solution three times a week maintained the seedlings in a fairly healthy condition. However, data from the experiments in which equivalent total amounts of iron were added daily and weekly to two sets of solutions indicated that most of the iron soon became unavailable, and that the weekly additions were spaced too far apart to accomplish the maximum effect of iron. It was found unnecessary to renew any of the nutrient solutions except iron during the experiment.

The deleterious effects upon the growth of wheat seedlings in Experiment X, caused by the addition of the solutions of tissue-free extracts of the pathogens used, are of interest because host invasion is not involved. Nevertheless, the very evident effect that the fungal extracts produce on the wheat plants may be viewed as an expression of pathogenicity. Further work is required to elucidate this important relationship.

References

1. BROADFOOT, W. C. and TYNER, L. E. Can. J. Research, C, 16 (6) : 253-261. 1938.
2. FISHER, R. A. Statistical methods for research workers. 5th ed. rev. and enl. Oliver and Boyd, Ltd., Edinburgh and London. 1934.
3. SHAFFER, P. A. and HARTMANN, A. F. J. Biol. Chem. 45 (2) : 349-364, 365-390. 1921.
4. SHIVE, J. W. Plant Physiol. 16 (3) : 435-445. 1941.
5. SNEDECOR, G. W. Calculation and interpretation of analysis of variance and covariance. Collegiate Press, Inc., Ames, Ia. 1934.

DIURNAL CHANGES IN THE CARBOHYDRATES OF WHEAT LEAVES[1]

By G. Krotkov[2]

Abstract

Data are presented on the diurnal variations in the sugars of wheat leaves, as well as on changes in the sugar content of detached and attached leaves, which were taken progressively during the 24 hr. and were starved under the adopted standardized conditions.

It was found that in some samples of leaves there was a considerable decrease in their sugars under the conditions of starvation, but on the other hand leaf samples taken either in the first part of a day, or after sunset, usually contained more sugars after starvation than before it. This last observation suggested a considerable hydrolysis of some complex substances in leaves, with the consequent appearance of sugars. From the analysis of the data obtained it was concluded that the observed diurnal variations in the extent of hydrolysis, synthesis, or in translocation of sugars are greatly influenced by the conditions of illumination.

It was reported previously (3) that, in the initial stages of starvation of detached wheat leaves, sugars serve as their respiratory substrate. On the basis of this observation it appeared possible to determine for wheat leaves a balance sheet for the metabolism and translocation of their sugars. The technique of experimentation should be as follows.

From a population of wheat plants three samples of leaves are taken simultaneously, and one of these is analysed for the initial sugar content of leaves. Two others, one consisting of detached and the other of attached leaves, are starved for a short period of time under similar conditions, and then are analysed for their final sugar content. The total amounts of carbon dioxide produced by the detached leaves in starvation are also determined.

The amounts of sugars lost in respiration are calculated from the amounts of carbon dioxide evolved. The difference between the initial sugar content of leaves and that of detached leaves after starvation represents the amounts of sugars lost both in respiration and in the transformation of sugars into some other organic substances (synthesis). Knowing the amounts of sugars lost in respiration one can calculate the amounts lost in synthesis. On the assumption that respiration and synthesis are taking place at the same rate in both detached and attached leaves, the difference in the sugar content between these two kinds of leaves after starvation is due to the translocation of sugars.

By taking a series of such samples throughout the consecutive 24 hr. one can obtain the diurnal changes in such a balance sheet, and by using leaves of different ages, changes during their development. The experiments described

[1] *Manuscript received October 19, 1942.*

Contribution from the Department of Biology, Queen's University, Kingston, Ont., with financial assistance from the National Research Council of Canada and the Science Research Committee of Queen's University.

[2] *Assistant Professor of Biology.*

in this paper were conducted with the object of obtaining such a balance sheet for wheat, and of exploring how it changes both diurnally and with the ontogeny of leaves.

Materials and Methods

Experiments 1 and 2 were carried out in the summer of 1938, and Experiments 3, 4, and 5 in the summer of 1940. A pure line of Marquis wheat seeds was used as experimental material. Plants were grown in the University greenhouse, and no attempts have been made to regulate environmental conditions during their growth.

On May 10, 1938, 250 5-in. pots were filled with well mixed sandy loam soil, and 20 seeds were put in each pot. The pots were watered, and left covered with paper until the appearance of seedlings above the ground, when the paper was removed.

Samples for Experiment 1 were taken when plants were seven days old from the time of sowing. At this age each plant had one foliage leaf only. These leaves were still increasing in length, their respiration rate was very high, and endosperm still contained starch. According to Duff and Forward (1) leaves with such characteristics are in the juvenile stage of their ontogeny.

Following sampling for Experiment 1 the rest of the population of plants was left growing in the greenhouse. The new foliage leaves, which appeared later, were clipped off with scissors every few days. In this way each plant was always represented by the first foliage leaf, which was passing through different physiological stages of its ontogeny unaffected by the successive leaves.

Samples of leaves for Experiment 2 were taken from plants that were 20 days old from the time of sowing. At this age leaves have completed their elongation, their respiration rate has dropped considerably, and starch has gone from the remnants of the endosperm. According to Duff and Forward (1) leaves with such characteristics are in the mature stage of their ontogeny.

Plants for Experiments 3, 4, and 5 were grown in a manner similar to that described for Experiments 1 and 2. The seeds were sown on May 8, 1940, and leaf samples were taken of plants 7, 13, and 21 days old, respectively.

On the day previous to the taking of the samples for each experiment, all the available plants were examined, and those belonging to the upper or lower quadrants according to their height were removed. The remaining homogeneous population of plants provided the stock from which samples were taken all the next day. Sampling for each experiment was begun 61 min. + 11 S.D. before sunrise, and was continued for 24 hr. with sampling periods approximately every three hours.

The first step in each sampling period was to cut 100 leaves at the ligule. One-half of these constituted a subsample for the determination of the initial sugar content of leaves, while the other half formed a subsample of detached leaves. The whole procedure of cutting 100 leaves occupied less than 10 min.,

and the time at which this was begun has been taken as the zero time for each particular sampling period.

The subsample of 50 leaves for the determination of the initial sugar content was weighed and extracted with 85% ethyl alcohol. The period between the zero time and the time they were dropped into boiling alcohol was 39 minutes ± 12 S.D. The technique of extraction, and of the subsequent analysis of the alcoholic extracts, was described in an earlier paper (3).

The subsample of detached leaves was weighed, and its carbon dioxide production was determined for a period of 208 min. ± 26 S.D., while the leaves were kept in darkness at 20 ± 0.25° C. At the end of their starvation, the leaves were extracted with alcohol, and their sugar content determined. Observations on the carbon dioxide production were begun 28 min. ± 8 S.D. after the zero time. The continuous air current method was used for the determination of the carbon dioxide evolved by the leaves (3).

The subsample of attached leaves was represented by plants growing in four pots. These pots were taken from the greenhouse, placed in a dark incubator at 20 ± 0.25° C., and kept there for a period of 191 min. ± 32 S.D. Then 50 leaves were cut from these plants, weighed, extracted with alcohol,

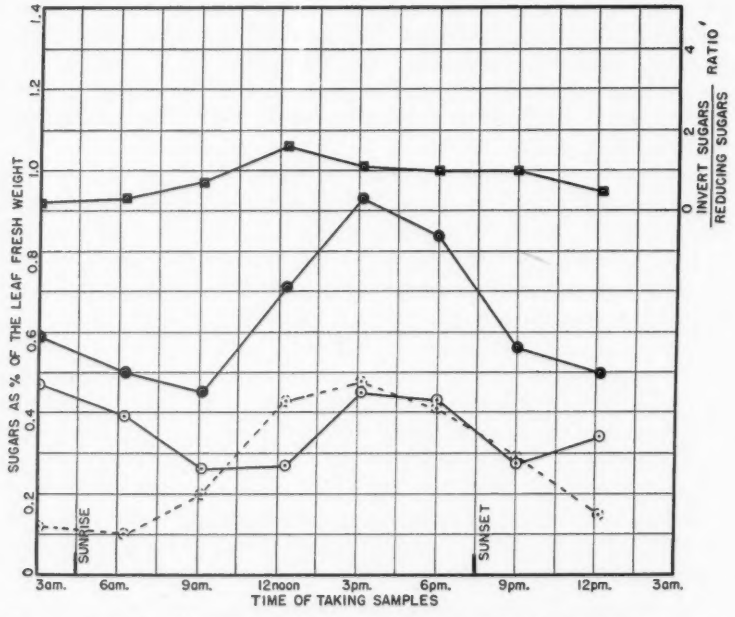

FIG. 1. *Expt. 1. Diurnal changes in the initial sugar content of wheat leaves;* ⊙- - - - - ⊙ *invert sugars,* ⊙——⊙ *reducing sugars,* ●——● *total sugars,* ■——■ $\frac{\text{invert sugars}}{\text{reducing sugars}}$ *ratio.*

and their sugar content was determined. The period between the time that pots were placed in a dark incubator and the zero time was 13 min. ± 14 S.D.

Results

Diurnal changes in the initial sugar content of leaves are given in Figs. 1 to 5. An examination of these figures reveals that total sugars increase during the day reaching a considerable peak in the afternoon between 3 p.m. and 6 p.m., and then decline again. These diurnal changes are quite regular in Experiments 1, 2, and 4, which were carried out on warm days with continuous sunshine. Deviations from this regularity, observed in Experiments 3 and 5, are easily traced to weather conditions. There were several showers during the day on which Experiment 3 was carried out. The day of Experiment 5 was cold, cloudy, and without any sunshine.

Of the total sugars, reducing and invert sugars are the two fractions that were determined, and their absolute and relative amounts show definite changes both diurnally and with the advancing age of leaves. In young leaves the reducing sugar content is relatively high, and its considerable diurnal variations are essentially of the same kind as those for the total sugars. In older leaves the reducing sugar content declines in its absolute amount,

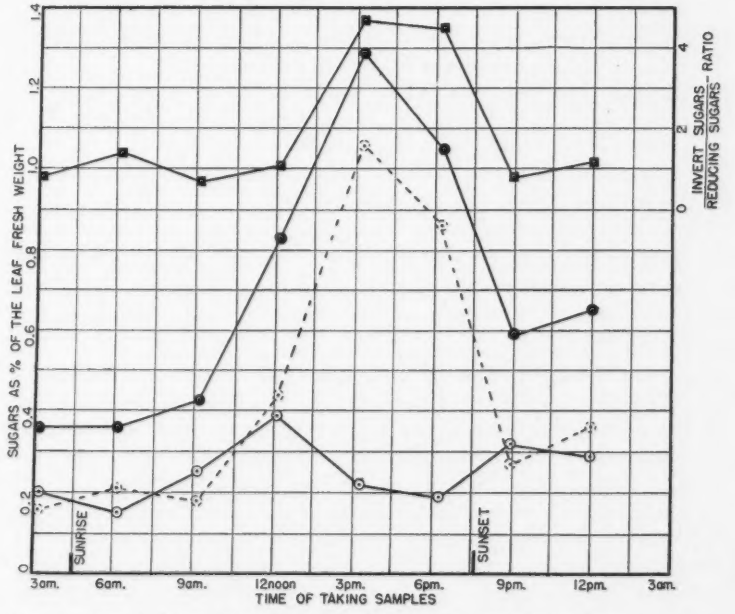

FIG. 2. *Expt. 2. Diurnal changes in the initial sugar content of wheat leaves;* ⊙------⊙ *invert sugars,* ⊙——⊙ *reducing sugars,* ●——● *total sugars,* ■——■ $\frac{\text{invert sugars}}{\text{reducing sugars}}$ *ratio.*

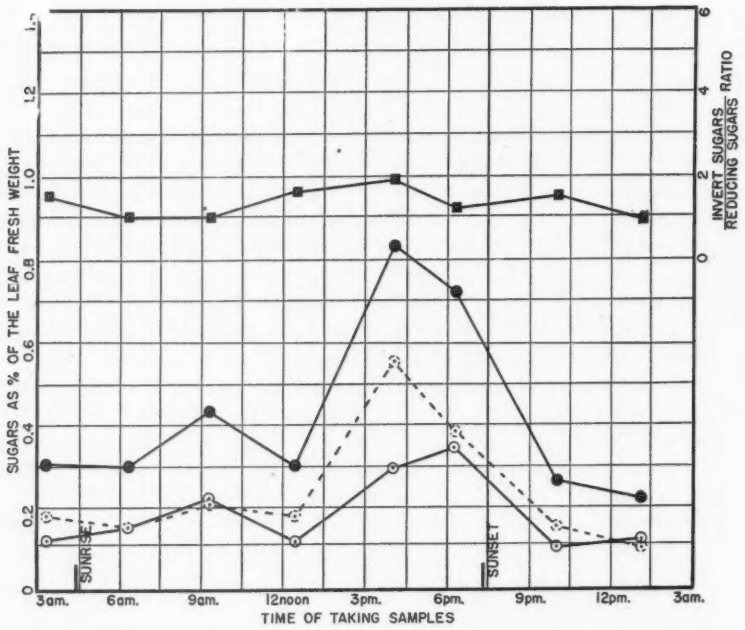

FIG. 3. *Expt. 3. Diurnal changes in the initial sugar content of wheat leaves;* ⊙------⊙ *invert sugars,* ⊙——⊙ *reducing sugars,* ●——● *total sugars,* ■——■ $\frac{\text{invert sugars}}{\text{reducing sugars}}$ *ratio.*

and becomes more constant during the 24 hr. On the other hand, in two out of three experiments performed on older leaves an increase is observed both in the absolute amounts of their invert sugars, as well as in the magnitude of their diurnal variations. The unfavourable weather during Experiment 5 may easily account for the deviation from the usual tendency observed in the third experiment.

Figs. 6 and 7 give changes in the respiration of leaves during the 24 hr. While diurnal fluctuations in the respiration of leaves at first sight appear to be similar to those of total sugars, on careful examination they are seen to have some important points of difference. The peaks of both graphs do not coincide in time, and the highest value for respiration during the day always precedes that for the total sugars by a few hours. In addition, a second rise in respiration occurs either shortly after sunset, or later in the night. Such an increase in respiration may occur either simultaneously with a rise in reducing or in invert sugars, or even when both these sugar fractions are decreasing.

In order to explore the relationship between respiration and sugar content, respiration rates of leaves have been plotted against their total sugars. Fig. 8

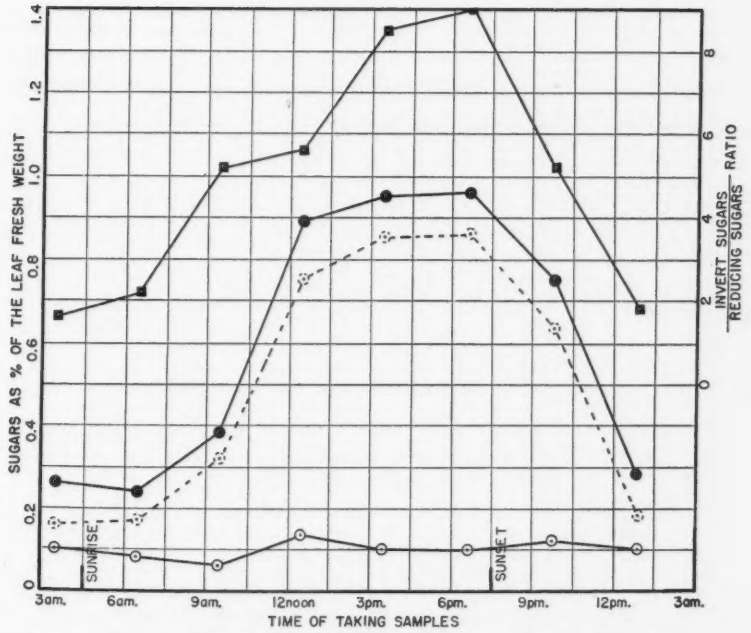

FIG. 4. *Expt. 4. Diurnal changes in the initial sugar content of wheat leaves;* ⊙- - - - - - ⊙ *invert sugars,* ⊙——⊙ *reducing sugars,* ●——● *total sugars,* ■——■ $\frac{\text{invert sugars}}{\text{reducing sugars}}$ *ratio.*

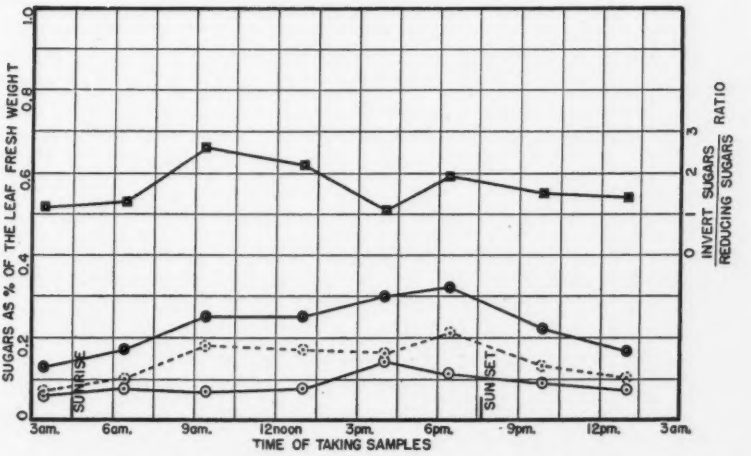

FIG. 5. *Expt. 5. Diurnal changes in the initial sugar content of wheat leaves;* ⊙- - - - - - ⊙ *invert sugars,* ⊙——⊙ *reducing sugars,* ●——● *total sugars,* ■——■ $\frac{\text{invert sugars}}{\text{reducing sugars}}$ *ratio.*

presents such a relationship for Experiment 1. From the examination of this figure it becomes evident that the direct proportionality between respiration and sugar content is only approximate. To facilitate further analysis of

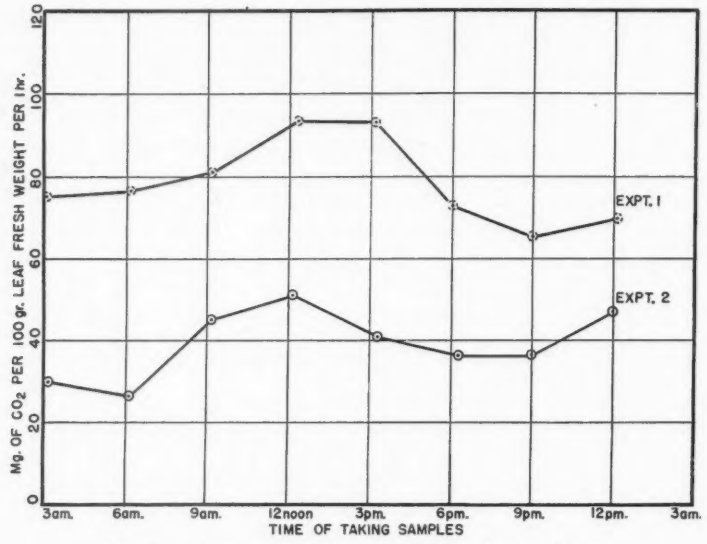

FIG. 6. *Diurnal changes in the respiration of wheat leaves.*

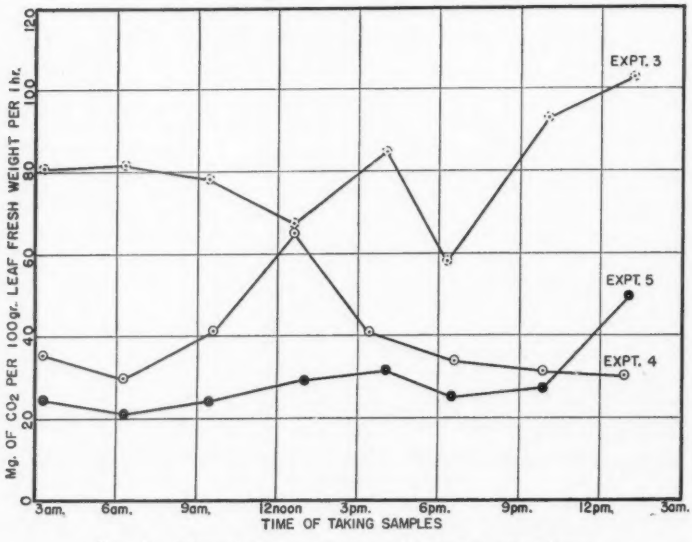

FIG. 7. *Diurnal changes in the respiration of wheat leaves.*

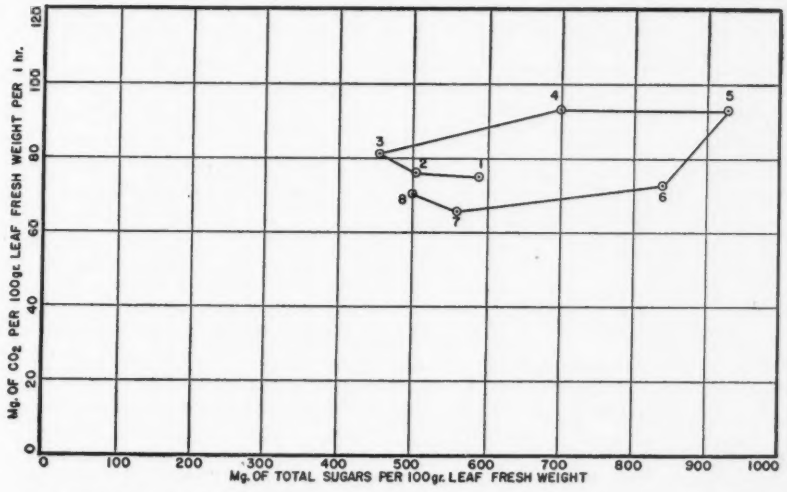

FIG. 8. *Expt. 1. Diurnal changes in the relation between respiration and total sugar content of wheat leaves.*

these data, respiration rates of various samples have been numbered in their chronological order, and then connected by a line. In this way the order of taking various samples also becomes evident.

All the respiration rates appear now to lie on a circle in their chronological order. Taking the relationship between respiration and total sugar content of Sample 1 for the starting point, it is seen that in Samples 2 and 3 respiration is higher in spite of their decreased sugar content. In Sample 4 both respiration and sugar content are increased, but in Sample 5 a still further increase in sugars occurs without any corresponding effect on respiration. In Samples 6 and 7 both respiration and sugars go down, but a still further decrease in the sugar content of Sample 8 is accompanied not by a decrease, but by an increase in respiration. Similar results are obtained for the rest of the experiments, or when respiration rates are plotted not against the total but against either reducing or invert sugar contents of leaves.

It is clear from Fig. 8 that the efficiency of sugars, as the respiratory substrate of wheat leaves, is increased early in the day and later in the night. Their lowest efficiency is early in the afternoon. An explanation of this variation of their efficiency in time might be that the chemical nature of both reducing and invert sugar fractions of wheat leaves is not the same through the 24 hr. In order to press further analysis of the relationship between respiration and sugar content of leaves, one should have a better knowledge as to the nature of the sugars present.

Fig. 9 gives for Experiment 1 the initial total sugar contents of various leaf samples, as well as the total sugars present in the respective subsamples

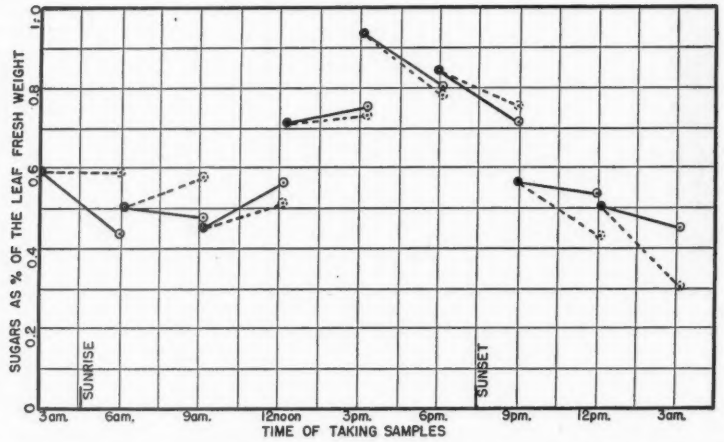

FIG. 9. *Expt. 1. Diurnal changes in the initial total sugar content of leaves, and in the corresponding subsamples of detached and attached leaves after 3 hr. of starvation;* ● *initial sugar content,* - - - - - -⊙ *attached leaves,* ————⊙ *detached leaves.*

of detached and attached leaves after 3 hr. of starvation. Examination of this figure reveals that the initial total sugar content of Sample 1 was 0.588%. During the 3 hr. of starvation the detached leaves of this sample lost 155 mg. of their total sugars per 100 gr. of the initial leaf fresh weight. From this amount 153 mg. were lost in respiration, leaving the difference of 2 mg. for the synthesis.

The initial total sugar content of Sample 2 was 0.505%. During the 3 hr. of starvation the detached leaves of this sample lost 29 mg. of their total sugars per 100 gr. of the initial leaf fresh weight. Since at the same time they also lost 157 mg. of sugars in respiration, one has to conclude that there must have been some addition of sugars, from some source, to these leaves during their starvation. But the amounts of sugars added apparently were not large enough to account for the whole of respiration, and, consequently, leaves had to withdraw for that purpose 29 mg. from the total sugars that they contained initially. The magnitude of this addition of sugars was expressed by the difference between the initial and the final sugar content of detached leaves, plus the amounts of sugars lost in respiration.

In Sample 3, even without a correction for respiration, the total sugar content of detached leaves was higher after starvation that it was before. Apparently during the starvation of detached leaves of this sample, sugars were also added to leaves. The magnitude of this addition can be calculated in a manner similar to that described above for Sample 2. Similar results were obtained in the rest of the samples of Experiment 1, and in various samples of Experiments 2, 3, 4, and 5.

The simplest explanation as to the source of the sugars added during the starvation of detached leaves is that they are produced as a result of hydrolysis

of some complex, insoluble substances present in leaves. That wheat leaves contain several kinds of such substances had been shown earlier (4). In the absence of better analytical methods, the absolute amounts of sugars added to detached, starving leaves can be taken as a measure of the extent of hydrolysis of these complex substances. Some of the sugars, produced as result of hydrolysis, may be used at once for the synthesis of other organic substances. For this reason their absolute amounts do not necessarily represent the whole magnitude of hydrolysis, but may stand only for its excess above the simultaneous synthesis. In a similar manner a value obtained for the synthesis may not represent anything more than its excess over simultaneous hydrolysis.

As stated in the introduction, the experiments described in this paper were conducted for the purpose of obtaining a balance sheet for the metabolism and translocation of sugars in wheat leaves. The proposed evaluation of the magnitude of synthesis was based on the assumption that under the conditions of starvation sugars must always disappear from detached leaves, and the amounts lost must be at least equal to those used in respiration. An observation that considerable amounts of sugars can be added to detached leaves in starvation, precludes the use of the proposed method for the determination of the full magnitude of synthesis in leaves. Some better methods for this purpose must be devised before the desired sugar balance sheet can be obtained.

But the results of the experiments, while falling short of their original object, reveal the existence of not only synthetic, but also of the hydrolytic activity in leaves. Figs. 10, 11, 12, 13, and 14 give data for all the experiments on the diurnal changes of such hydrolysis and synthesis. An examination of these figures reveals that the hydrolytic and synthetic activities of leaves undergo a series of definite diurnal variations. Throughout the first part of a day there is a general increase in hydrolysis, with a peak reached sometime between 9 a.m. and 4 p.m. In every case this peak occurs shortly before the total sugar content of leaves has reached its highest value. Then hydrolysis declines, while synthesis increases, with its peak occurring usually late in the afternoon, though in Experiment 4 this peak is not reached until shortly after sunset. In the night there is another rise in hydrolysis.

At first sight diurnal changes in the hydrolysis and synthesis of Experiment 3 appear to have followed a somewhat different course from the general one described above. In this experiment there are two peaks in hydrolytic activity during the day instead of one. These two peaks are followed, as in the rest of the experiments, by an increase in synthesis later in the day, and by a rise in hydrolysis during the night. But examination of the graph for the total sugar content of these leaves shows that during the day there are also two peaks in their total sugar content instead of the usual one. And, as in the rest of the experiments, both peaks in the hydrolytic activity of these leaves occur shortly before those in their total sugars. In other words, even these unusual changes in the hydrolytic activity of leaves in Experiment 3 early in the day bear the same relationship to the total sugar contents of leaves, as found in the rest of the experiments.

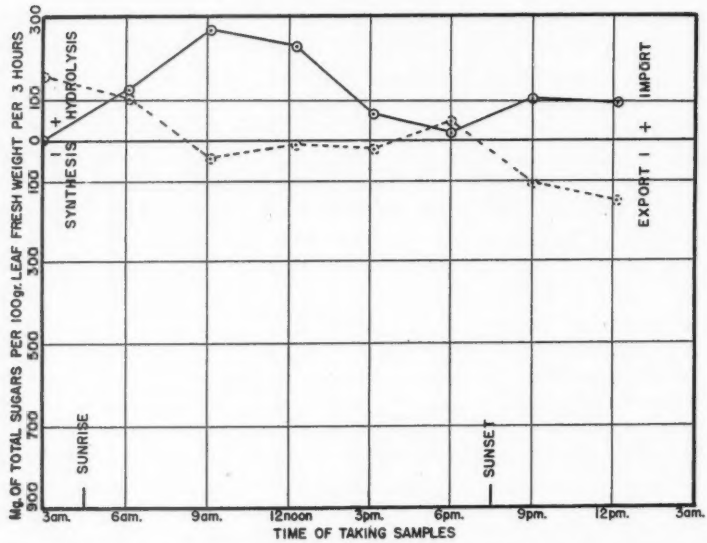

FIG. 10. *Expt. 1. Diurnal changes in the hydrolysis–synthesis and in the translocation of sugars in wheat leaves;* ⊙————⊙ *hydrolysis–synthesis,* ⊙- - - - -⊙ *translocation of sugars.*

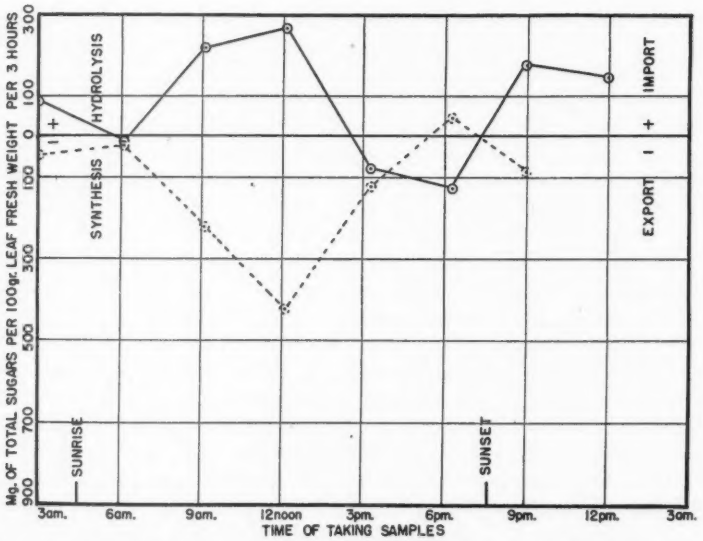

FIG. 11. *Expt. 2. Diurnal changes in the hydrolysis–synthesis and in the translocation of sugars in wheat leaves;* ⊙————⊙ *hydrolysis–synthesis,* ⊙- - - - -⊙ *translocation of sugars.*

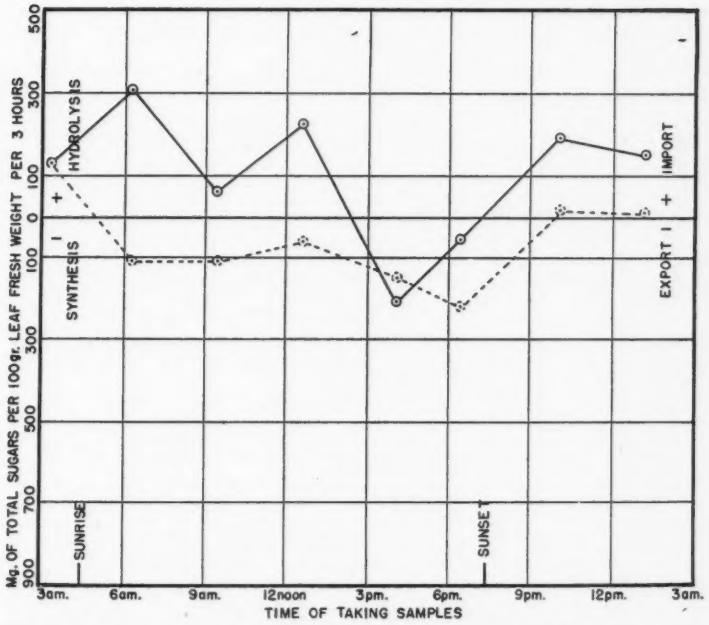

FIG. 12. *Expt. 3. Diurnal changes in the hydrolysis–synthesis and in the translocation of sugars in wheat leaves;* ⊙————⊙ *hydrolysis–synthesis,* ⊙- - - - -⊙ *translocation of sugars.*

These diurnal variations in the hydrolytic and synthetic activity of leaves appear to be closely related to the conditions of illumination. That light may have such an effect has been reported by several workers. Lubimenko and Karisnev (6), growing wheat and barley seedlings under different conditions of illumination, found the greatest dry weight of plants and the lowest respiration under intermediate conditions of illumination, and not in full sunlight or in darkness. They concluded that the intensity of illumination has a direct effect on the synthetic, as well as on the oxidative processes in a living, green tissue. That the effect of light on enzymes may be different, depending on the light intensity, has been reported at least for one of the hydrolytic enzymes —diastase (2).

In the present work the peak in synthesis occurs usually in the afternoon, when conditions of illumination are intermediate. In four experiments out of five, there is observed either a decrease in hydrolysis, or even a slight synthesis, early in the forenoon, when conditions of illumination are also intermediate.

A prolonged illumination of leaves during a day results in a change from hydrolysis to synthesis. This change is undoubtedly due at least partly to a change in the light intensity. But a low value for hydrolysis before sunrise

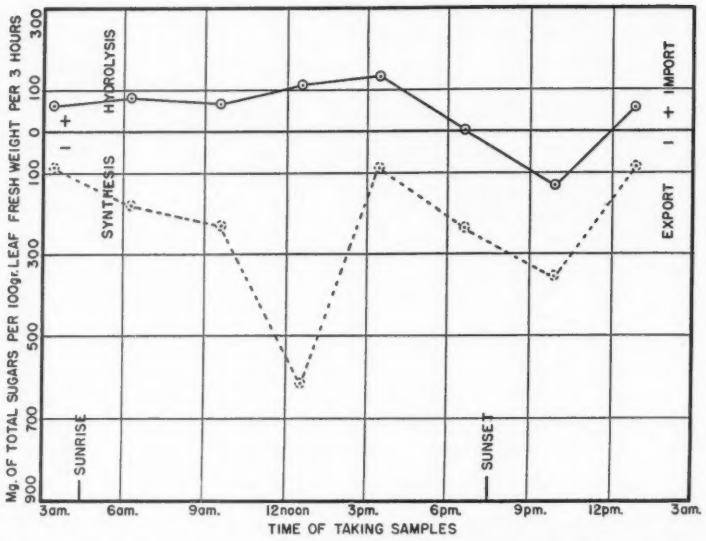

FIG. 13. *Expt. 4. Diurnal changes in the hydrolysis–synthesis and in the translocation of sugars in wheat leaves;* ⊙————⊙ *hydrolysis–synthesis,* ⊙- - - - -⊙ *translocation of sugars.*

suggests that the stimulating effect of darkness on hydrolysis is only temporary. In other words, the length of the period of darkness, and perhaps also of illumination, is a factor affecting hydrolysis. Lepeschkin (5) concluded from the study of yeast cells that the first effect of direct sunlight on living matter is to decrease its stability. Such a decrease in stability, however, is only temporary, and it disappears completely in two hours in spite of continuous illumination.

All the evidence given above suggests that not only the intensity of illumination, but also the length of the period of illumination or of darkness, may determine whether hydrolytic or synthetic activity will prevail in a cell.

The diurnal changes in the translocation of sugars from attached leaves are also given in Figs. 10, 11, 12, 13, and 14. A careful examination of these figures shows that translocation does not take place at a constant rate throughout the 24 hr., but undergoes a series of definite diurnal fluctuations. Just before sunrise the flow of sugars from the leaves is either very low, or in some cases may be reversed. From the beginning of illumination there is a general increase in export, and the main peak is reached sometime between 9 a.m. and 6 p.m. Then export of sugars declines again, and near sunset sugars may even be imported into the leaves. At night there may occur another increase in export. The peak in the export of sugars never coincides with that of the total sugar content of leaves. In four experiments out of five, the peak in export occurred a few hours earlier.

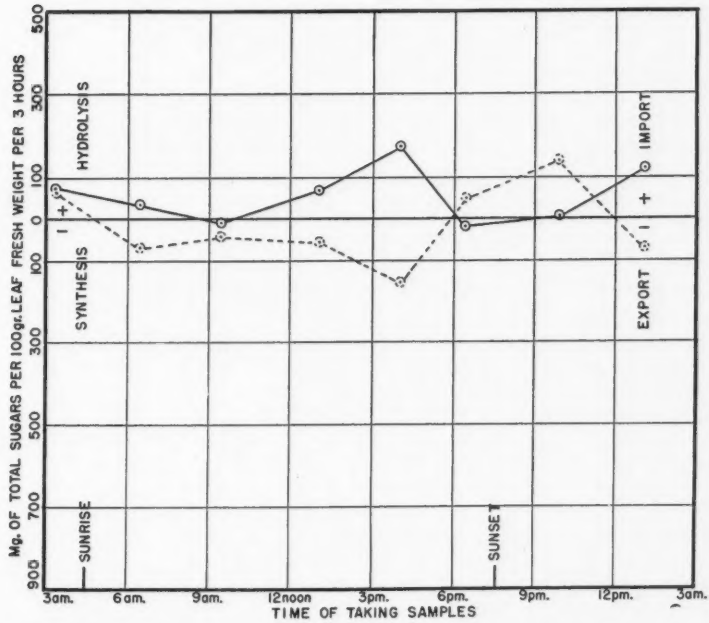

FIG. 14. *Expt. 5. Diurnal changes in the hydrolysis–synthesis and in the translocation of sugars in wheat leaves;* ⊙————⊙ *hydrolysis–synthesis,* ⊙- - - - - -⊙ *translocation of sugars.*

A comparison of the graph for the diurnal changes in the hydrolytic and synthetic activities of leaves with that for the translocation of sugars suggests a close relationship between the two. In Experiments 1, 2, and 5 these two graphs are complementary one to another. A high rate of hydrolysis coincides with a high rate of sugar export. When hydrolysis is at its lowest value, or synthesis is at its peak, sugars are either imported into the leaves, or at least their export is very low. In other words, when sugars in leaves are released, their export is considerable; when sugars are bound, they are imported.

The results of Experiments 3 and 4 indicate, however, that such a close relationship between the hydrolysis–synthesis and the translocation of sugars in leaves does not occur in every case. The general trend of diurnal changes for both of these graphs is the same in all five experiments. But while in Experiments 3 and 4 the graphs for the hydrolysis–synthesis and translocation begin to diverge early in the day, as in the rest of the experiments, later in the day their peaks fail to synchronize in time. As a consequence, graphs begin to run more or less parallel one to another. Either in Experiments 3 and 4 there appeared some new factor later in the day, which modified the usual timing of events, or these two processes are affected by light independently one from another. In the last case, a definite relationship between the

hydrolysis–synthesis, and the translocation of sugars observed in Experiments 1, 2, and 5, is a pure coincidence.

Acknowledgments

The author wishes to express his thanks to the U.S. Bureau of Plant Industry for its kindness in supplying the seeds, and to Mr. C. Jamieson and Mr. V. A. Helson for their technical help in connection with this work.

References

1. DUFF, G. H. and FORWARD, D. F. Paper read before the Biological Section, Royal Society of Canada, May, 1932. (*Abstracted in* Proc. Roy Soc. Can. (Ser. 3) 26 (Appendix B): CVII–CVIII. 1932.)
2. HUTCHINSON, A. H. and ASHTON, M. R. Can. J. Research, 9 (1) : 49-64. 1933.
3. KROTKOV, G. Plant Physiol. 14 (2) : 203-226. 1939.
4. KROTKOV, G. Plant Physiol. 14 (3) : 559-565. 1939.
5. LEPESCHKIN, W. W. Am. J. Botany, 19 (7) : 547-558. 1932.
6. LUBIMENKO, V. and KARISNEV, R. Compt. rend. acad. sci. U.R.S.S., Ser. A, No. 22 : 381-386. 1927.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 21, SEC. D. JANUARY, 1943 NUMBER 1

DRIED WHOLE EGG POWDER

I. METHODS OF ASSESSING QUALITY[1]

BY M. W. THISTLE[2], J. A. PEARCE[2], AND N. E. GIBBONS[3]

Abstract

Several methods of assessing quality in dried whole egg powders were studied on a wide range of material from Canadian egg drying plants. Moisture content and bacterial count varied independently, and are regarded as necessary measures of quality. Beating value, pH of egg batter, water value, potassium chloride value, fluorescence measurements, and palatability ratings were all significantly interrelated. Of these methods, potassium chloride value and fluorescence measurements were the most sensitive and also the most closely associated with palatability ratings.

Introduction

The necessity of transporting eggs to Britain in the dried form, in order to supply a less perishable product than shell eggs and also to save shipping space, has resulted in an investigation of methods of estimating and controlling the quality of the first-class dried whole egg powders required for domestic consumption. This paper describes and assesses the methods of measuring quality that appeared to be useful when the work was undertaken. Methods other than those described are under test.

Materials

The relative suitability of the various methods for assessing quality could best be studied on samples showing a wide range of quality. However, it was also desirable to meet the conditions of a practical test. To fulfil these requirements, two sets of samples were obtained, prime quality powders secured weekly from five Canadian plants for a period of six weeks, and a range of inferior material consisting of 19 residue samples, obtained from the secondary dust collectors of four of the plants.

Methods

All methods of assessing quality in egg powder that appeared to be useful when this study was undertaken were used concurrently on the 49 samples. Details of these tests follow.

[1] *Manuscript received October 17, 1942.*

Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as paper No. 84 of the Canadian Committee on Food Preservation and as N.R.C. No. 1097.

[2] *Biochemist, Food Investigations.*

[3] *Bacteriologist, Food Investigations.*

Moisture as Total Volatiles

The method used was a modification of a standard A.O.A.C. procedure (1, p. 308): duplicate 10-gm. samples were heated in a vacuum oven at 100° C. for 12–16 hr. or overnight. These conditions suited laboratory practice, and it was found that the extra time did not materially affect the results.

Bacterial Count

Five grams of powder was ground in a mortar with 10 gm. of sand and 45 ml. of water to give a homogeneous emulsion. After dilution, aliquots were plated in duplicate on a medium composed of 0.5% proteose–peptone (Difco), 0.5% tryptone (Difco), and 1.5% agar. The pH was adjusted to 6.8–7.0 if necessary. Counts were made after three days' incubation at 37° C.

Counts were also made after seven days' incubation at 20° C., but as they were similar to three-day counts at 37° C., the longer incubation period was discontinued.

Beating Value

The method was a modification of that in use at the Low Temperature Research Station, Cambridge, England*. Eighteen grams of egg powder was mixed with 60 gm. of sugar, placed in a small "Mixmaster" bowl, and reconstituted by adding 57 ml. of distilled water and mixing at the lowest speed of the mixer for five minutes, followed by 20 min. mixing at the highest speed. The volume of the resulting material constituted the beating value.

pH on Residue from Beating Test

Rather than make separate solutions for pH measurements, it appeared advisable to use left-over solutions from other tests. Materials from the water value, potassium chloride value, and beating tests have all been used, but in this particular series use was made of the residue from the beating test.

Measurements were made with the Beckman pH meter, using a glass electrode and a saturated calomel half-cell.

Water Value

It may be interjected at this point that water value and potassium chloride value (to be discussed) take into account possible protein–lipoid associations, and therefore should not be confused with those solubility tests that are concerned with protein only.

An accurately weighed portion of about 2.2 gm. of powder was shaken for an hour in 100 ml. of distilled water, and filtered through a No. 1 Whatman filter paper. An aliquot of 20 ml. of filtrate was transferred to a weighed beaker and coagulated on a hot-plate. The coagulated material was then dried at 110° C. for 16 hr., and the residue weighed and expressed as a percentage of the original weight of sample.

* *Private communication.*

Potassium Chloride Value

The procedure was the same as that described for the water value, except that 10% potassium chloride solution replaced water. The weight of dissolved salt was subtracted, and the residual weight expressed as a percentage of the weight of sample taken.

Fluorescence Measurements

Briefly, 2.5 gm. of defatted egg powder (fat extracted with chloroform) was shaken with 10% potassium chloride, and the fluorescence of the extract measured with a Coleman photofluorometer. Details of the method have been described (2).

Palatability Test

Details have also been given for this test (2). Each sample, mixed with water and coagulated in a water-bath, was tasted by a panel of 13 people. The original British system of scoring was followed, ranging from 10 for excellent, fresh egg to 0 for repulsive material. Recently more elaborate work on the palatability test has been published (3).

Results

Mean values for all measurements on material from different plants are given in Table I. Fluorescence measurements were more precise, and distinguished more sharply between powders from different plants than the other quality tests. Potassium chloride value is also indicated as being among the more sensitive tests.

TABLE I

MEAN VALUES OF QUALITY MEASUREMENTS ON EGG POWDERS FROM DIFFERENT PLANTS

Groups tested	Plant No.	Methods							
		Volatile materials, %	Bacterial count, log	Beating value, ml.	pH	Water value, %	KCl value, %	Fluorescence, units	Palatability, score
Prime quality samples	1	4.41	4.75	237	8.36	76.9	70.7	19.2	7.35
	2	4.35	5.80	260	8.43	75.9	75.5	15.9	8.03
	3	3.27	5.35	235	8.44	74.7	76.9	19.2	7.58
	4	5.70	6.27	251	8.31	73.4	75.5	18.8	7.71
	5	4.39	4.01	258	8.45	71.8	81.1	19.9	7.56
Necessary difference[1]		0.05	0.69	*n.s.d.*[2]	*n.s.d.*[2]	1.8	1.6	0.2	*n.s.d.*[2]
Dust-collector samples	1	3.08	4.67	249	8.23	71.1	63.1	30.0	6.11
	2	4.12	5.15	173	7.94	54.4	38.4	46.8	5.17
	3	3.49	5.57	160	7.90	51.2	33.7	50.9	5.20
	4	5.78	6.24	250	8.25	73.9	69.0	21.0	7.18
Necessary difference[1]		0.17	0.72	39	0.23	4.8	3.4	1.7	0.63

[1] *Necessary difference at the 5% level of significance.*

[2] *No significant difference between plant averages on prime quality samples.*

The significance of the over-all quality differences between plants and between sampling times was assessed by means of analyses of variance, given in Tables II and III. The objective tests, shown in Table II, generally distinguished average differences between plants on prime samples, whereas palatability ratings did not, as shown in Table III. On dust-collector materials, on the other hand, all tests showed marked differences between plant averages.

TABLE II

ANALYSES OF VARIANCE OF QUALITY IN EGG POWDERS, AS ASSESSED BY OBJECTIVE TESTS

Groups tested	Variance attributable to:	Degrees of freedom	Mean square						
			Volatile materials	Bacterial count	Beating value	pH	Water value	Potassium chloride value	Fluorescence measurements
Prime quality samples	Plants	4	8.90**	4.70**	818	0.021	48.9**	165.4**	29.6**
	Samplings	5	2.92**	0.45	593	0.025	214.2**	74.7**	48.5**
	Plants × samplings	20	2.55**	0.33	294	0.010	31.3**	35.0**	17.1**
	Error between duplicates	30	0.0042				4.8	3.6	0.047
Dust-collector samples	Plants	3	14.09**	2.23**	11766**	0.168*	1300.4**	2962.5**	1975.0**
	Samplings	4	0.44*	0.40	1169	0.060	341.0**	770.5**	189.2**
	Plants × samplings	11	1.35**	0.36	1048	0.037	134.6**	214.8**	149.8**
	Error between duplicates	19	0.033				26.1	13.0	3.394

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

TABLE III

ANALYSES OF VARIANCE OF QUALITY IN EGG POWDERS, AS ASSESSED BY A FLAVOUR PANEL

Groups tested	Variance attributable to:	Degrees of freedom	Mean square
Prime quality samples	Plants	4	2.96
	Samplings	5	9.79**
	Plants × samplings	20	2.75
	Replicates	12	3.86*
	Error	335	1.92
Dust-collector samples	Plants	4	44.14**
	Samplings	3	19.55**
	Plants × samplings	12	12.23**
	Replicates	12	7.58**
	Error	200	2.64

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

Since palatability is a necessary judgment of desirability of foodstuffs, correlation of objective measurements with palatability is regarded as an important factor in the choice of methods. Analyses of covariance (Table IV) show that the two distinct sets of data, for prime quality and for dust-collector

TABLE IV

ANALYSES OF COVARIANCE OF PALATABILITY AND OTHER QUALITY MEASUREMENTS

Source of variance	Degrees of freedom	Mean square						
		Palatability and volatile materials	Palatability and bacterial count	Palatability and beating value	Palatability and pH	Palatability and water value	Palatability and KCl value	Palatability and fluorescence
Average regression	1	6.07**	1.34	35.20**	35.27**	32.03**	46.84**	49.20**
Differences in position	1	25.75**	29.60**	8.68**	3.56*	8.06**	0.81	0.53
Differences in slope	1	2.90*	0.38	1.00	4.91**	1.66	0.60	0.28
Residual	45	0.70	0.77	0.47	0.50	0.54	0.39	0.36

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

samples, may be combined for purposes of correlating palatability with fluorescence, and palatability with potassium chloride value. These two relations, therefore, are the only ones given in the general correlation table (Table V) under the grouping of prime quality and dust-collector samples.

Coefficients of correlation between the methods of assessing quality in dried egg powders are shown in Table V. On prime quality samples, beating value and pH were both mildly associated with potassium chloride value; pH was also correlated with fluorescence measurements. The slight degree of association between bacterial count and palatability was presumably fortuitous, since it was unconfirmed on the wider range of quality in dust-collector samples. However, all correlation values are low, and the general lack of correlation, considered in conjunction with palatability ratings, suggests that there was no great difference in quality between the prime samples.

The correlation coefficients between measurements made on residue material, from the secondary dust-collectors, showed that increase in moisture content was mildly associated with an increase in bacterial count. There is also some indication that a low moisture content was associated with a high fluorescence reading. Both these relations probably reflect plant practice. The six remaining measures of quality were all highly interrelated. All correlated to a somewhat higher degree with fluorescence values than with any other measurement, though an almost equal association was shown between palatability and potassium chloride value. Correlation of objective tests with palatability fell into three classes: moisture content and bacterial count

TABLE V

COEFFICIENTS OF CORRELATION BETWEEN VARIOUS QUALITY MEASUREMENTS

Groups tested	Methods	Methods						
		Volatile materials	Bacterial count	Beating value	pH	Water value	KCl value	Fluorescence
Prime quality samples	Bacterial count	.276						
	Beating value	−.038	−.030					
	pH	−.153	−.209	.127				
	Water value	.023	.113	−.177	.096			
	KCl value	−.130	−.086	.362*	.410*	.252		
	Fluorescence	−.125	−.327	−.254	−.498**	.186	−.229	
	Palatability	.170	.355*	.226	.024	.177	.248	−.310
Dust-collector samples	Bacterial count	.577*						
	Beating value	.293	.199					
	pH	.229	.025	.719**				
	Water value	.341	.103	.744**	.747**			
	KCl value	.406	.250	.834**	.726**	.800**		
	Fluorescence	−.511*	−.266	−.868**	−.851**	−.883**	−.913**	
	Palatability	.464	.266	.747**	.730**	.675**	.819**	−.854**
Prime quality and dust-collector samples	Palatability						.841**	−.866**

* *Exceeds 5% level of significance.*
** *Exceeds 1% level of significance.*

were not associated with palatability; correlations between palatability and beating value, pH, and water value all occupied an intermediate position; potassium chloride value and fluorescence measurements correlated most highly with palatability.

Discussion

Potassium chloride value and water value must not be confused with the results of soluble nitrogen determinations. Nitrogen values are concerned with protein only, operate at a much higher and narrower range of percentages, and were found to consume too much time and material to be of practical benefit in routine control work. Potassium chloride value and water value represent the amount of material passing through No. 1 Whatman filter paper. Since dried egg contains approximately 40% fat, ordinarily insoluble in water or potassium chloride solutions the highest solubility to be expected in these tests would be about 60% of the original sample. In practice, however, higher values are observed, indicating the presence of egg oil in the filtrate. This behaviour can best be explained by the assumption that some combination of lipoid and protein, emulsion or otherwise, exists in the natural yolk, and carries the fat through the filter paper. Processing or storage treatments apparently alter this complex, resulting in low values by the procedures used. The range and sensitivity of the potassium chloride values obtained in this study lend considerable support to the above hypothesis, and suggest

that this empirical measurement may be more useful as a measure of quality than methods designed to determine the exact solubility of a particular constituent.

No correlation with palatability score was shown by any objective measurement on prime quality powders over the narrow range between 7.3 and 8.1 on the palatability scale. Of the methods studied here, the best objective measure of quality was fluorescence, correlating with palatability to the level of −0.854 on powders with a wide range of quality. However it is held that the difficulty on prime quality samples lies with the palatability test and not with fluorescence. Independent confirmation of this view is forthcoming from work now in progress in these laboratories. Very good powder has been lowered in quality, by a series of time–temperature treatments, to a level where a taste panel can distinguish the difference: within this range, fluorescence values establish a whole series of points, corresponding exactly with the time–temperature treatments. This indicates both the selectivity of the fluorescence method, and the insensitivity of panel ratings.

A more experienced panel may provide the more precise data necessary to establish correlation over the narrow high-quality range, and this possibility is being investigated.

Conclusions

Of the methods studied, moisture content, bacterial count, and one or more of the remaining tests are necessary for control purposes. Moisture content and bacterial count are independent measures of quality, contributing necessary information. Present evidence indicates that moisture content should be as low as is compatible with high quality in other respects. Bacterial counts yield information on the general condition of both the liquid egg and the powder produced. Of the remaining tests, fluorescence measurements and potassium chloride value are considered to be the most suitable, as they were more sensitive to minor differences in quality, and were associated with palatability to a closer extent than were the other methods studied. On the bases of selectivity and ease of operation, fluorescence is indicated as being the best single choice. However, since the correlation coefficients between potassium chloride value and palatability, and between fluorescence measurements and palatability did not differ significantly, the potassium chloride value may be useful if a fluorometer is not available.

Acknowledgments

The authors wish to express their appreciation of the assistance rendered by Mr. W. Harold White, Biochemist, and Dr. J. W. Hopkins, Statistician.

References

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis. 5th ed. A.O.A.C., Washington, D.C. 1940.
2. Pearce, J. A. and Thistle, M. W. Can. J. Research, D, 20(9) : 276-282. 1942.
3. Wilson, R. V. and Slosberg, H. M. Food Industries, 14(9) : 56-58. 1942.

THE EFFECT OF FREEZING AND THAWING ON THE QUALITY OF CANNED HERRING[1]

By F. Charnley[2] and O. C. Young[3]

Abstract

The effect of freezing and thawing on the quality of canned herring is investigated by comparing averages of the quality characteristics: firmness, volume of free aqueous liquid, and volume of free oil of treated samples with those of untreated samples. Repeated freezing and thawing definitely impairs the quality of canned herring by causing absorption of an appreciable proportion of the free aqueous liquid and free oil into the interior of the sample and seriously diminishing the firmness of the cooked tissue.

The investigation reported in this paper was undertaken to determine the magnitude of the changes in quality of canned herring arising through repeated freezing and thawing treatments. During the winter months, parcels of British Columbia canned herring that are shipped across Canada may, under certain circumstances, be subjected to one or more freezing and thawing cycles. Such a process, if accompanied by large temperature changes, will manifestly affect the quality of the canned product, but the extent of such changes in quality of canned products of this type does not appear to have been investigated hitherto. Accordingly, before it could be decided whether or not shipments of canned herring should be made in protected cars, it was necessary to ascertain the magnitudes of these changes in quality, as indicated by several easily measured quality characteristics of canned herring.

Treatment of Samples

The general plan of the experiments was very simple, namely, to submit samples of canned herring to several different freezing and thawing cycles and to compare the averages of the quality characteristics of the treated samples with those of untreated samples. In carrying out the experiments, however, it was found necessary to take into account three complicating factors. The first of these was that the quality characteristics of canned herring show considerable variation from sample to sample. Secondly, certain characteristics of this product are subject to pronounced seasonal variations, and, thirdly, it was necessary to allow for the effect of handling on the quality of the samples in the shipment of the experimental samples from Vancouver, B.C., to the Fisheries Experimental Station, Prince Rupert, B.C., and thence back again to the Canned Fish Inspection Laboratory, Vancouver, B.C.

The effect of the first and second of these factors was overcome by subjecting samples of 12 cans to the different treatments and comparing the

[1] *Manuscript received in original form April 7, 1942, and as revised, August 3, 1942.*
Contribution from the Canned Fish Inspection Laboratory, Vancouver, B.C., and the Pacific Fisheries Experimental Station, Prince Rupert, B.C.

[2] *Chief Chemist, Canned Fish Inspection Laboratory, Vancouver, B.C.*
[3] *Associate Research Engineer, Pacific Fisheries Experimental Station, Prince Rupert, B.C.*

treated samples with a control sample of 12 cans of the same code, that is, with samples packed by the same cannery on the same day, while possible differences arising from the third factor were avoided by shipping the control samples, along with the samples to be treated, to the Fisheries Experimental Station, where the freezing and thawing experiments were conducted, and likewise returning the control samples along with the treated samples to the Canned Fish Inspection Laboratory, where the samples were tested and the changes in quality determined.

The experimental freezing and thawing cycles to which the samples were subjected were divided into three different treatments, samples of 12 1-lb. ovals and 12 1-lb. talls being subjected to each of the three treatments. These treatments were as follows.

(A). The samples were placed in an environment of approximately minus 22° C. and were removed 23 hr. later, when the centres of the cans had reached a temperature of minus 19° C. as determined by means of an automatic recorder and thermocouples inserted in additional 1-lb. oval and 1-lb. tall cans. The two sets of samples were then removed from the refrigerated chamber and allowed to stand in the basement of the building at a temperature of approximately 15° C. After about 15 hr. the temperature of the centres of the cans had risen to 14° C. The samples were then subjected to a second freezing cycle under the same conditions as the first for a period of about 28 hr., when the centres of these cans, as determined by thermocouples in adjacent cans, had reached a temperature of minus 22° C. The samples were again transferred to the basement of the building and attained the temperature of this room (15° C.) slightly over 17 hr. later. At the completion of the experiment on December 1 the samples were packed in shipping cases and forwarded to Vancouver, B.C.

(B). The samples were subjected to two freezing and thawing cycles as in (A) except that the first freezing was to a temperature of minus 16° C., the first thawing to 14° C., and the second freezing to minus 14° C.

(C). The samples were subjected to two freezing and thawing cycles as in (A) except that the freezing was to minus 10° C. in each case and the thawing was to 14° C.

The control samples consisting of 12 1-lb. oval and 12 1-lb. tall cans were stored in the basement of the building during the experiments and, as mentioned above, were returned under the same shipping conditions as the treated samples.

The three rates of cooling were arbitrarily chosen, since it was not known through what conditions a transcontinental shipment of herring might pass in midwinter. In any type of railway car that might be employed, the freezing would certainly be slow, and for those cans in the centre of the load the cooling would obviously be slower than for those on the outside. Consequently, in these experiments different rates and degrees of cooling were employed to ascertain whether or not these variables were significant in producing changes

in the quality of the canned product. In all three treatments the cans were merely placed in still air, the temperature of which was controlled.

Freezing and thawing curves corresponding to the three different treatments are shown in Fig. 1. These curves were contracted from the recorder charts, upon which the temperature at the centre of any particular can was recorded every eight minutes, and illustrate graphically the relations between the temperature of the centres of 1-lb. tall cans and the time in hours from the commencement of the experiment. As will be observed from the figure, the time of cooling in the first cycle was approximately 23 hr. and the time of warming was about 15 hr., while the cooling and warming periods in the second cycle were respectively 28 and 17 hr.

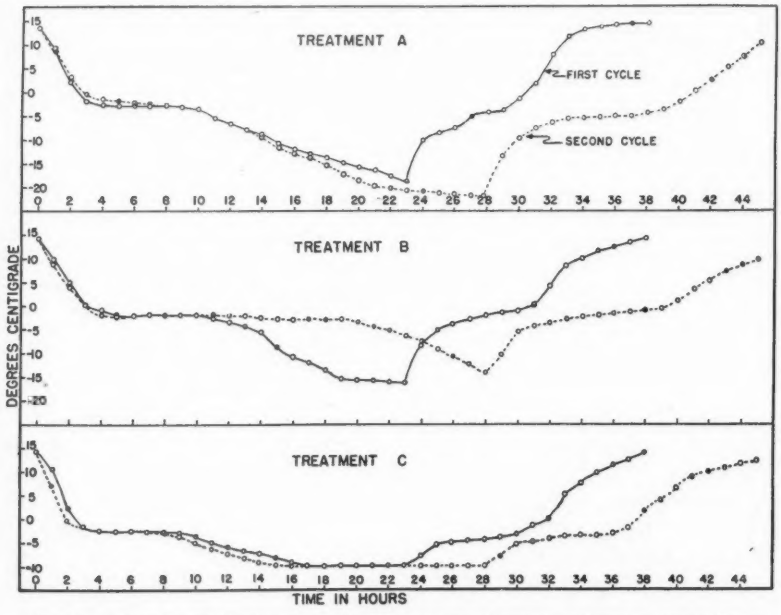

FIG. 1. *Freezing and thawing curves for 1-lb. tall cans of herring subjected to three different treatments.*

Examination of Samples

Changes in the quality of the samples resulting from the freezing and thawing treatments were investigated by measuring the following quality characteristics on individual samples: firmness of the fish tissue, volume of free aqueous liquid, volume of free oil, pH of free aqueous liquid, and refractive index of oil at 25° C. The last two of these characteristics, however, that is, the pH of the aqueous liquid and the refractive index of the oil, were not appreciably affected by the freezing and thawing treatments, possibly because, on the one hand, the addition of tomato sauce to the samples masked the

true pH values, and on the other hand, the free oil represents only a part of the total fat content of the samples, and would, furthermore, tend to exist in contact with only a portion of the surface of the solid tissue. Quality changes due to the freezing and thawing treatments were thus more satisfactorily determined by means of the first three characteristics.

In measuring firmness, values of the depth of penetration shown by an Armstrong penetrometer (1) were first obtained by taking averages of three readings on 1-lb. oval samples and single readings on 1-lb. tall samples. The values of depth of penetration were then converted into measures of firmness by means of the data (2) given in Table I. The advantages of the latter transformation are: *(a)* the resulting measure of firmness is a measure of the resistance of the sample to penetration; *(b)* the firmness increases in numerical value as the quality with respect to this characteristic improves; and *(c)* the measure of firmness is normally distributed, in contrast with depth of penetration which is highly skewed and leptokurtic (1).

TABLE I

CORRESPONDING VALUES OF MEASURE OF FIRMNESS AND DEPTH OF PENETRATION AT FIVE SECONDS

Depth of penetration, mm.	Firmness	Depth of penetration, mm.	Firmness	Depth of penetration, mm.	Firmness
4	24.9	12	7.8	20	3.7
5	21.0	13	6.9	21	3.4
6	17.9	14	6.2	22	3.2
7	15.3	15	5.6	23	3.0
8	13.2	16	5.1	24	2.9
9	11.4	17	4.7	25	2.7
10	10.0	18	4.3	30	2.1
11	8.8	19	4.0	35	1.7

The volumes of free aqueous liquid and free oil were measured by allowing the sample to drain for 15 or 20 min. into a graduate cylinder and reading off the respective volumes of the settled liquids at room temperature.

Typical results for the three characteristics, firmness, volume of free aqueous liquid, and volume of free oil are shown in Tables II and III, which give the results of the tests on the 1-lb. oval herring samples subjected to treatment B and on the 1-lb. oval samples used as controls.

Analysis of Results

Since there were three freezing and thawing treatments and two can sizes, the total number of measurements available for the estimation of the variance of each quality characteristic corresponding to a given can size was 48. A

TABLE II

RESULTS OF TESTS ON 1-LB. OVAL HERRING SAMPLES SUBJECTED TO TREATMENT B

Can No.	Depth of penetration, mm.	Vol. of free aq. liquid, cc.	Vol. of free oil, cc.	Can No.	Depth of penetration, mm.	Vol. of free aq. liquid, cc.	Vol. of free oil, cc.
1	11.0	23	3	7	16.3	18	3
2	11.7	8	1.5	8	18.0	14	4
3	11.7	5	3	9	14.7	14	3
4	13.0	0	1	10	13.7	18	3
5	16.0	17	5	11	13.3	10	1.5
6	12.3	16	4	12	12.3	26	7

TABLE III

RESULTS OF TESTS ON 1-LB. OVAL HERRING SAMPLES USED AS CONTROLS

Can No.	Depth of penetration, mm.	Vol. of free aq. liquid, cc.	Vol. of free oil, cc.	Can No.	Depth of penetration, mm.	Vol. of free aq. liquid, cc.	Vol. of free oil, cc.
1	10.7	14	3	7	9.7	22	5
2	14.7	13.5	3	8	12.7	17	2.5
3	13.3	21.5	4	9	15.0	23	4.5
4	11.3	29	3.5	10	13.0	13.5	3
5	12.3	32.5	6	11	11.0	19	3.5
6	12.0	26	3.5	12	11.3	19	7

simple and, at the same time, flexible method of evaluating the results, therefore, was to estimate variances from the pooled results for each can size and to determine the significance of the differences in means by converting the latter into t values. Where necessary, it was then a relatively simple matter by utilizing the fact that the differences in freezing and thawing treatment resulted in no observable differences in quality to increase the sensitiveness of the comparisons by combining the results derived from the treated samples.

Tables IV to IX give the details of the estimation of the variances of the individual quality characteristics corresponding to given can sizes, together with the t values corresponding to the differences in means between the treated and control samples. Application of Pearson's formula (4), namely,

$$\sigma_c^2 = \frac{1}{N - k} \sum_{i=1}^{k} (n_i s_i^2)$$

to the totals of the columns headed "ns^2" gives the estimates of the variances of the individual quality characteristics shown at the bottom of Tables IV to IX. The variance of the difference in two averages of 12 (σ_d^2) required in calculating the t values is evidently 1/6 of the estimated variance given in the tables.

TABLE IV

ESTIMATION OF VARIANCE OF FIRMNESS OF 1-LB. OVAL HERRING SAMPLES AND CALCULATION OF t VALUES

Treatment	n	ns^2	Mean	Difference in means, d	t value
A	12	8.1622	5.8750	−1.8667	−3.9827
B	12	21.7566	6.6833	−1.0584	−2.2582
C	12	6.1491	5.4917	−2.2500	−4.8005
Control	12	21.9691	7.7417		
Total	48	58.0370		$\sigma_e^2 = 1.3190$	$\sigma_d = 0.4687$

NOTE: *5% values of t: for $n = 30$, $t = 2.042$; for $n = \infty$, $t = 1.960$.*

TABLE V

ESTIMATION OF VARIANCE OF VOLUME OF FREE AQUEOUS LIQUID OF 1-LB. OVAL HERRING SAMPLES AND CALCULATION OF t VALUES

Treatment	n	ns^2	Mean	Difference in means, d	t value
A	12	430.06	17.1250	−3.7083	−1.4616
B	12	598.92	14.0833	−6.7500	−2.6605
C	12	258.73	14.7917	−6.0416	−2.3813
Control	12	411.67	20.8333		
Total	48	1699.38		$\sigma_e^2 = 38.622$	$\sigma_d = 2.5371$

NOTE: *5% values of t: for $n = 30$, $t = 2.042$; for $n = \infty$, $t = 1.960$.*

TABLE VI

ESTIMATION OF VARIANCE OF VOLUME OF FREE OIL OF 1-LB. OVAL HERRING SAMPLES AND CALCULATION OF t VALUES

Treatment	n	ns^2	Mean	Difference in means, d	t value
A	12	6.562	3.3750	−0.6667	−1.3933
B	12	29.750	3.2500	−0.7917	−1.6545
C	12	3.916	2.9167	−1.1250	−2.3511
Control	12	20.229	4.0417		
Total	48	60.457		$\sigma_e^2 = 1.3740$	$\sigma_d = 0.4785$

NOTE: *5% values of t: for $n = 30$, $t = 2.042$; for $n = \infty$, $t = 1.960$.*

TABLE VII

ESTIMATION OF VARIANCE OF FIRMNESS OF 1-LB. TALL HERRING SAMPLES AND CALCULATION OF t VALUES

Treatment	n	ns^2	Mean	Difference in means, d	t value
A	12	77.8860	4.2333	−2.3000	−3.1949
B	12	23.9622	4.4250	−2.1083	−2.9286
C	12	13.3621	4.8250	−1.7083	−2.3730
Control	12	21.6065	6.5333		
Total	48	136.8168		$\sigma_e^2 = 3.1095$	$\sigma_d = 0.7199$

NOTE: *5% values of t: for $n = 30$, $t = 2.042$; for $n = \infty$, $t = 1.960$.*

TABLE VIII

ESTIMATION OF VARIANCE OF VOLUME OF FREE AQUEOUS LIQUID OF 1-LB. TALL HERRING SAMPLES AND CALCULATION OF t VALUES

Treatment	n	ns^2	Mean	Difference in means, d	t value
A	12	1793.23	38.2917	−13.375	−2.8058
B	12	969.42	31.4167	−20.250	−4.2480
C	12	1099.67	41.1667	−10.500	−2.2027
Control	12	2136.67	51.6667		
Total	48	5998.99		$\sigma_e^2 = 136.340$	$\sigma_d = 4.7669$

NOTE: *5% values of t: for $n = 30$, $t = 2.042$; for $n = \infty$, $t = 1.960$.*

TABLE IX

ESTIMATION OF VARIANCE OF VOLUME OF FREE OIL OF 1-LB. TALL HERRING SAMPLES AND CALCULATION OF t VALUES

Treatment	n	ns^2	Mean	Difference in means, d	t value
A	12	61.062	11.3750	−5.1250	−5.1204
B	12	74.729	11.2917	−5.2083	−5.2036
C	12	59.667	10.8333	−5.6667	−5.6616
Control	12	69.000	16.5000		
Total	48	264.458		$\sigma_e^2 = 6.0104$	$\sigma_d = 1.0009$

NOTE: *5% values of t: for $n = 30$, $t = 2.042$; for $n = \infty$, $t = 1.960$.*

From an inspection of Tables VII, VIII, and IX, it will be seen that the 1-lb. tall herring samples, which were subjected to the freezing and thawing treatments, differ very significantly from the control samples as regards firmness, volume of free aqueous liquid, and volume of free oil, and, as shown in Table IV, there are similar significant differences in firmness between the treated and control 1-lb. oval herring samples. In fact, in these four sets of data the differences in the averages are so pronounced that it is not necessary to combine the results derived from the treated samples to demonstrate the presence of significant effects. The lowest t value (2.2027) in these four sets would occur by chance less than four times in 100, while t values approximating 2.5 and 3 are associated with probabilities that are less than 0.02 and 0.01 respectively. There is thus no doubt in these four sets of data of very definite changes in quality due to the freezing and thawing treatments.

In contrast with the results of the 1-lb. tall samples, the t values of Tables V and VI corresponding to differences in the free aqueous liquid and free oil of the 1-lb. oval samples are not all individually significant. The t values associated with treatments B and C in Table V, and treatment C in Table VI are individually significant, but the remaining values are not individually conclusive. The fact that the values are all of the same sign, however, readily suggests that significant differences would be obtained on comparing the results of the tests on the control samples with the combined results derived from the treated samples.

This inference is found to be correct. Comparison of the variance between treated samples with the variance within samples, as summarized in Tables X and XI, shows that the treated samples may safely be considered homogeneous as regards the characteristics, free aqueous liquid and free oil. The values of z are, respectively, -0.1199 and -0.3562 each with $n_1 = 44$ and $n_2 = 2$ degrees of freedom. The 5% point, however, for $n_1 = \infty$ and $n_2 = 2$ is, from Fisher's (3) table, $z = 1.4851$, so that, as judged by these data, there is no evidence of lack of homogeneity in the samples, and consequently, no evidence of any difference in quality resulting from the differences in freezing and thawing treatment.

TABLE X

ANALYSIS OF VARIANCE OF FREE AQUEOUS LIQUID IN 1-LB. OVAL HERRING SAMPLES

Variance	D.f.	Sum of squares	Mean square	Log$_e$ s.d.
Between treated and control samples	1	272.25	272.25	2.8034
Between treated samples	2	60.79	30.39	1.7070
Within samples	44	1699.38	38.62	1.8269
Total	47	2032.42		

TABLE XI

ANALYSIS OF VARIANCE OF FREE OIL IN 1-LB. OVAL HERRING SAMPLES

Variance	D.f.	Sum of squares	Mean Square	Log$_e$ s.d.
Between treated and control samples	1	6.674	6.674	0.9491
Between treated samples	2	1.347	0.674	−0.1973
Within samples	44	60.457	1.374	0.1589
Total	47	68.478		

Accordingly, we may legitimately combine the treated samples and compare the averages of the combined results with the averages given by the control samples. From Table V the average free aqueous liquid for the treated samples is 15.3333. The difference in average free aqueous liquid in the treated and control samples is therefore 5.5000 with variance 1/9 of the estimated variance. Hence the value of t is 2.654 with 44 degrees of freedom. Similarly, the corresponding value of t for the data of Table VI is 2.203 with the same number of degrees of freedom. Consequently, the differences between the treated and control 1-lb. oval herring samples with respect to volume of free aqueous liquid and volume of free oil are also significant.

From an examination of the data listed in Tables IV to IX, it will be observed that the variances of the treated samples differ considerably in a number of treatments from those of the control samples. The variances corresponding to treatments A and C of Tables IV and VI, and treatment B of Table VIII for example, are apparently much lower than the corresponding variances in the control samples, and the reverse is true of the variances of treatment A of Table VII. It could therefore be contended that the resulting t values have been largely influenced by the differences in the variances and that, as a consequence of this, the differences in the means are not necessarily significant.

That the means themselves differ significantly, however, is readily demonstrated by taking advantage of the fact that all the differences are of the same sign and applying a test appropriate to this circumstance. If, for example, the control samples of the 1-lb. oval herring data are segregated into groups of four and each of these groups is associated with a corresponding treatment, the averages shown in Table XII are obtained. From these results it is seen that the differences in the averages between the treated and control samples are in all except one instance negative, and in the one exception the difference is zero. Since, by virtue of the method of packing, all these characteristics are substantially independent, it follows that, even if the magnitudes of the differences in the averages are neglected, the probability of obtaining the eight minus signs is $(1/2)^8 = \frac{1}{256}$ under the hypothesis that the means do not differ significantly. There is thus no doubt that the freezing and thawing treatments have definitely impaired the quality of these samples.

TABLE XII

COMPARISON OF AVERAGES OF FOUR CONTROL SAMPLES WITH AVERAGES OF TREATED 1-LB. OVAL HERRING SAMPLES

Treatment	Firmness		Free aqueous liquid		Free oil	
	Treated	Control (Av. of 4)	Treated	Control (Av. of 4)	Treated	Control (Av. of 4)
A	5.875	7.550	17.125	19.500	3.375	3.375
B	6.683	8.225	14.083	24.375	3.230	4.250
C	5.492	7.450	14.792	18.625	2.917	4.500

The fact that the volume of free aqueous liquid and volume of free oil of the 1-lb. oval herring samples are relatively less affected by the freezing and thawing treatments than these characteristics of the 1-lb. tall herring samples is apparently connected with the difference in the methods of processing the two can sizes. In packing oval cans part of the liquids formed is usually drained off, whereas these liquids are usually retained in packing 1-lb. tall cans.

Conclusion

From the foregoing results it must, therefore, be concluded that repeated freezing and thawing definitely impairs the quality of canned herring. Freezing and thawing of this product apparently causes considerable changes in the fish muscle tissue with absorption of an appreciable proportion of the free aqueous liquid and free oil into the interior of the sample, thus resulting in a serious diminution in the firmness of the cooked tissue.

Acknowledgments

The writers acknowledge with thanks the valuable assistance rendered by other members of the staffs of the Pacific Fisheries Experimental Station and the Canned Fish Inspection Laboratory in carrying out the above investigation. To Dr. N. M. Carter, Director, Mr. E. P. Sidaway, Scientific Assistant in Research Engineering, and Mr. Geo. Baker, Junior Technician, of the Fisheries Experimental Station, thanks are due for very substantial assistance in carrying out the freezing and thawing experiments, while similar thanks are extended to Mr. R. S. Bolton, Mr. F. R. E. Davies, Miss P. C. Cumming, and Mrs. O. Volkoff, Assistants at the Canned Fish Inspection Laboratory, for their work in carrying out the tests on the experimental samples, and also a number of the calculations in the preparation of the paper. Finally, sincere thanks are due to the reviewers of the manuscript for a number of very helpful criticisms in the statistical treatment of the data and abbreviation of the tables.

References

1. CHARNLEY, F. and BOLTON, R. S. J. Fisheries Research Board Can. 4(3) : 162-173. 1938.
2. CHARNLEY, F. and BOLTON, R. S. Unpublished results.
3. FISHER, R. A. Statistical methods for research workers. 5th ed. rev. and enl. Oliver and Boyd, Ltd., Edinburgh and London. 1934.
4. PEARSON, E. S. J. Roy. Stat. Soc. (n.s.) 96(1) : 21-60; discussion, 60-75. 1933.

CANADIAN JOURNAL OF RESEARCH

Notes on the Preparation of Copy

General:—Manuscripts should be typewritten, double spaced, and the *original* and *one copy* submitted. Style, arrangement, spelling, and abbreviations should conform to the usage of this Journal. Names of all simple compounds, rather than their formulae, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed. Manuscripts should be carefully checked before being submitted, to reduce the need for changes after the type has been set. All pages should be numbered.

Abstract:—An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required.

Illustrations

(i) *Line Drawings:*—Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper ruled in *blue* only. Paper ruled in green, yellow, or red should not be used. The principal co-ordinate lines should be ruled in India ink and all lines should be of sufficient thickness to reproduce well. Lettering and numerals should be of such size that they will not be less than one millimetre in height when reproduced in a cut three inches wide. If means for neat lettering are not available, lettering should be indicated in pencil only. All experimental points should be carefully drawn with instruments. Illustrations need not be more than two or three times the size of the desired reproduction, but the ratio of height to width should conform with that of the type page. The *original drawings* and one set of small but clear *photographic copies* are to be submitted.

(ii) *Photographs:*—Prints should be made on glossy paper, with strong contrasts; they should be trimmed to remove all extraneous material so that essential features only are shown. Photographs should be submitted *in duplicate;* if they are to be reproduced in groups, one set should be so arranged and mounted on cardboard with rubber cement; the duplicate set should be unmounted.

(iii) *General:*—The author's name, title of paper, and figure number should be written on the back of each illustration. Captions should not be written on the illustrations, but typed on a separate page of the manuscript. All figures (including each figure of the plates) should be numbered consecutively from 1 up (arabic numerals). Reference to each figure should be made in the text.

Tables:—Titles should be given for all tables, which should be numbered in Roman numerals. Column heads should be brief and textual matter in tables confined to a minimum. Reference to each table should be made in the text.

References should be listed alphabetically by authors' names, numbered in that order, and placed at the end of the paper. The form of literature citation should be that used in this Journal and titles of papers should not be given. All citations should be checked with the original articles. Each citation should be referred to in the text by means of the key number.

The *Canadian Journal of Research* conforms in general with the practice outlined in the *Canadian Government Editorial Style Manual*, published by the Department of Public Printing and Stationery, Ottawa.